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TITLE

PLANT CELLULOSE SYNTHASES

This application claims the benefit of U.S. Provisional Application No. 60/092,844, filed July 14, 1998.

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding cellulose biosynthetic enzymes in plants and seeds.

BACKGROUND OF THE INVENTION

Cellulose is a major component of plant fiber, e.g. cotton fiber. Cellulose is composed of crystalline beta-1,4-glucan microfibrils (see World Patent Publication No. WO 98/00549). These microfibrils are strong and can resist enzymatic and mechanical degradation and are important in determining nutritional quality of animal and human foodstuffs. Hence, modification of the biosynthetic pathway responsible for cellulose synthesis through modification of cellulose synthase activity could potentially alter fiber quantity, either by producing more or less fiber in a particular plant species or in a specific organ or tissue of a particular plant. Modification of cellulose synthase activity could increase the value of the fiber to the end-user and may improve the structural integrity of the plant cell wall. Lastly, because cellulose is a major cell wall component, inhibition of cellulose synthesis would probably be lethal. Thus, cellulose synthase may serve as the target for a novel class of herbicides. Plant cellulose synthase genes, homologs of the bacterial celA genes encoding the catalytic subunit of cellulose synthase, have been reported from cotton, *Arabidopsis*, rice and alfalfa (World Patent Publication Nos. WO 98/00549 and WO 98/18949).

There is a great deal of interest in identifying the genes that encode proteins involved in cellulose synthesis. These genes may be used in plant cells to control the synthesis of cellulose. Accordingly, the availability of nucleic acid sequences encoding all or a portion of a cellulose synthase would facilitate studies to better understand cellulose synthesis in plants and provide genetic tools to alter cellulose production.

SUMMARY OF THE INVENTION

The instant invention relates to isolated nucleic acid fragments encoding cellulose biosynthesis enzymes. Specifically, this invention concerns an isolated nucleic acid fragment encoding a cellulose synthase and an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding a cellulose synthase. In addition, this invention relates to a nucleic acid fragment that is complementary to the nucleic acid fragment encoding cellulose synthase. An additional embodiment of the instant invention pertains to a polypeptide encoding all or a substantial portion of a cellulose synthase.

In another embodiment, the instant invention relates to a chimeric gene encoding a cellulose synthase, or to a chimeric gene that comprises a nucleic acid fragment that is complementary to a nucleic acid fragment encoding a cellulose synthase, operably linked to suitable regulatory sequences, wherein expression of the chimeric gene results in production of levels of the encoded protein in a transformed host cell that is altered (i.e., increased or decreased) from the level produced in an untransformed host cell.

In a further embodiment, the instant invention concerns a transformed host cell comprising in its genome a chimeric gene encoding a cellulose synthase, operably linked to suitable regulatory sequences. Expression of the chimeric gene results in production of altered levels of the encoded protein in the transformed host cell. The transformed host cell can be of eukaryotic or prokaryotic origin, and include cells derived from higher plants and microorganisms. The invention also includes transformed plants that arise from transformed host cells of higher plants, and seeds derived from such transformed plants.

An additional embodiment of the instant invention concerns a method of altering the level of expression of a cellulose synthase in a transformed host cell comprising:
a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a cellulose synthase; and b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of altered levels of cellulose synthase in the transformed host cell.

An addition embodiment of the instant invention concerns a method for obtaining a nucleic acid fragment encoding all or a substantial portion of an amino acid sequence encoding a cellulose synthase.

A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of a cellulose synthase, the method comprising the steps of: (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a cellulose synthase, operably linked to suitable regulatory sequences; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of cellulose synthase in the transformed host cell; (c) optionally purifying the cellulose synthase expressed by the transformed host cell; (d) treating the cellulose synthase with a compound to be tested; and (e) comparing the activity of the cellulose synthase that has been treated with a test compound to the activity of an untreated cellulose synthase, thereby selecting compounds with potential for inhibitory activity.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE DESCRIPTIONS

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application.

Figure 1 shows a comparison of the amino acid sequences set forth in SEQ ID NOs:2, 4, 8, 10, 12, 14, 16, 18, 20 and 22 and the *Arabidopsis thaliana* sequences (SEQ ID NOs:23 (gi 2827139), 24 (gi 2827141), 26 (gi 4467125), 27 (gi 4886756) and 29 (gi 3135611)) and *Gossypium hirsutum* sequences (SEQ ID NOs:25 (gi 1706958) and 28 (gi 5081779)).

- 5 Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence
10 disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1
Cellulose Biosynthetic Enzymes

Protein	Clone Designation	SEQ ID NO:	
		(Nucleotide)	(Amino Acid)
Cellulose Synthase	bsh1.pk0002.f6	1	2
Cellulose Synthase	Contig composed of: cco1n.pk0005.g3 cdt2c.pk002.g1 cdt2c.pk002.l16 csc1c.pk002.i1 p0031.ccmr05rb p0110.cgsm57r	3	4
Cellulose Synthase	cr1n.pk0135.e10	5	6
Cellulose Synthase	p0097.cqrad17rc	7	8
Cellulose Synthase	p0122.ckamh70rc	9	10
Cellulose Synthase	rlr24.pk0073.g1	11	12
Cellulose Synthase	sdp2c.pk005.o22	13	14
Cellulose Synthase	ses8w.pk0028.f3	15	16
Cellulose Synthase	ssl.pk0036.c10	17	18
Cellulose Synthase	Contig composed of: wl1.pk0009.c9 wr1.pk0160.d11 wre1n.pk0043.f9 wre1n.pk0043.h8 wre1n.pk0131.g10	19	20
Cellulose Synthase	wl1n.pk0044.b1	21	22

- 15 The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The

symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. As used herein, a
5 “nucleic acid fragment” is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. A nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

As used herein, “contig” refers to a nucleotide sequence that is assembled from two or
10 more constituent nucleotide sequences that share common or overlapping regions of sequence homology. For example, the nucleotide sequences of two or more nucleic acid fragments can be compared and aligned in order to identify common or overlapping sequences. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be
15 assembled into a single contiguous nucleotide sequence.

As used herein, “substantially similar” refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. “Substantially similar” also refers to nucleic acid fragments wherein changes in
20 one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-suppression technology. “Substantially similar” also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting
25 transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof.

For example, it is well known in the art that antisense suppression and co-suppression
30 of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in
35 the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for

glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed
5 modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize, under stringent conditions (0.1X SSC, 0.1% SDS, 65°C), with the nucleic acid fragments disclosed herein.

10 Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Preferred are those nucleic acid fragments whose nucleotide
15 sequences encode amino acid sequences that are 80% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are 95% identical to the amino acid sequences reported herein. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite
20 (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

25 A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST
30 (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes
35 comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular

nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that
5 comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

10 "Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited
15 by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide building
20 blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. "Chemically synthesized", as related to nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments
25 may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards
30 those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its
35 own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived

from the same source, but arranged in a manner different than that found in nature.

“Endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign” gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A “transgene” is a gene that has been introduced into the genome by a transformation procedure.

“Coding sequence” refers to a nucleotide sequence that codes for a specific amino acid sequence. “Regulatory sequences” refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

“Promoter” refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an “enhancer” is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”. New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamoto and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

The “translation leader sequence” refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Molecular Biotechnology* 3:225).

The “3' non-coding sequences” refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences

encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell* 1:671-680.

5 “RNA transcript” refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. “Messenger RNA (mRNA)” refers to the RNA that is
10 without introns and that can be translated into polypeptide by the cell. “cDNA” refers to a double-stranded DNA that is complementary to and derived from mRNA. “Sense” RNA refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide by the cell. “Antisense RNA” refers to an RNA transcript that is
15 complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. “Functional RNA” refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

20 The term “operably linked” refers to the association of two or more nucleic acid fragments on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to
25 regulatory sequences in sense or antisense orientation.

 The term “expression”, as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. “Antisense inhibition” refers to the production of antisense RNA transcripts capable of
30 suppressing the expression of the target protein. “Overexpression” refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. “Co-suppression” refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

35 “Altered levels” refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

 “Mature” protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed.

“Precursor” protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

5 A “chloroplast transit peptide” is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. “Chloroplast transit sequence” refers to a nucleotide sequence that encodes a chloroplast transit peptide. A “signal peptide” is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53).
10 If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

15 “Transformation” refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as “transgenic” organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or “gene gun” transformation
20 technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference).

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989
25 (hereinafter “Maniatis”).

Nucleic acid fragments encoding at least a portion of a cellulose synthase enzyme have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be
30 used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase
35 chain reaction, ligase chain reaction).

For example, genes encoding other cellulose synthase enzymes, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing

methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673; Loh et al. (1989) *Science* 243:217). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165).

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol.* 36:1; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of cellulose synthase in those cells.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant chimeric gene can then be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. (1985) *EMBO J.* 4:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant polypeptides to different cellular compartments, or to facilitate its secretion from the cell. It is thus envisioned that the chimeric gene described above may be further supplemented by altering the coding sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell* 56:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.* 100:1627-1632) added and/or with targeting sequences that are already present removed. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of utility may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants for some applications. In order to accomplish this, a chimeric gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Alternatively, a chimeric gene designed to express antisense RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U. S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In addition, the ability to restrict the expression of specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above, it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on practical grounds, and is not an inherent part of the invention. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one which allows large numbers of samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired phenotype.

The instant polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptides. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded cellulose synthase. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 6).

Additionally, the instant polypeptides can be used as a targets to facilitate design and/or identification of inhibitors of those enzymes that may be useful as herbicides. This is desirable because the polypeptides described herein catalyze a step in the synthesis of cellulose. Accordingly, inhibition of the activity of one or more of the enzymes described
5 herein could lead to inhibition plant growth. Thus, the instant polypeptides could be appropriate for new herbicide discovery and design.

All or a substantial portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to those genes. Such information may be useful in plant
10 breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al.
15 (1987) *Genomics* 1:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map
20 previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet.* 32:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter* 4(1):37-41. Numerous publications describe genetic mapping of specific cDNA clones using the
25 methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used
30 for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask
35 (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan et al. (1995) *Genome Research* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 114(2):95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 5 16:325-332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nature Genetics* 7:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification 10 reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA* 86:9402; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* 92:8149; Bensen et al. (1995) *Plant Cell* 7:75). The latter approach may be 20 accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the 25 mutation tag element in or near the plant gene encoding the instant polypeptides. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a 30 mutation in the endogenous gene encoding the instant polypeptides can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

EXAMPLES

The present invention is further defined in the following Examples, in which all parts 35 and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without

departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

EXAMPLE 1

Composition of cDNA Libraries: Isolation and Sequencing of cDNA Clones

5 cDNA libraries representing mRNAs from various barley, corn, rice, soybean and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2
cDNA Libraries from Barley, Corn, Rice, Soybean and Wheat

Library	Tissue	Clone
bsh1	Barley (<i>Hordeum vulgare</i>) sheath, developing seedling	bsh1.pk0002.f6
cco1n	Corn (<i>Zea mays</i>) cob of 67 day old plants grown in green house*	cco1n.pk0005.g3
cdt2c	Corn (<i>Zea mays</i>) developing tassel 2	cdt2c.pk002.g1 cdt2c.pk002.116
cr1n	Corn (<i>Zea mays</i>) root from 7 day seedlings grown in light*	cr1n.pk0135.e10
csc1c	Corn (<i>Zea mays</i>) 20 day seedling (germination under cold stress)	csc1c.pk002.i1
p0031	Corn (<i>Zea mays</i>) shoot culture, initiated from seed derived meristems culture was maintained on 273N medium.	p0031.ccmr05rb
p0110	Corn (<i>Zea mays</i>) stages V3/V4** leaf tissue minus midrib harvested 4 hours, 24 hours and 7 days after infiltration with salicylic acid, tissues pooled*	p0110.cgsm57r
p0097	Corn (<i>Zea mays</i>) stage V9** whorl section (7 cm) from plant infected four times with european corn borer	p0097.cgrad17rc
p0122	Corn (<i>Zea mays</i>) pith tissue collected from internode subtending ear node 5 days after pollination	p0122.ckamh70rc
rlr24	Rice (<i>Oryza sativa</i>) leaf (15 days after germination) 24 hours after infection of <i>Magaporthe grisea</i> strain 4360-R-62 (AVR2-YAMO); Resistant	rlr24.pk0073.g1
sdp2c	Soybean (<i>Glycine max</i>) developing pods 6-7 mm	sdp2c.pk005.o22
ses8w	Soybean (<i>Glycine max</i>) mature embryo 8 weeks after subculture	ses8w.pk0028.f3
ssl	Soybean (<i>Glycine max</i>) seedling 5-10 day	ssl.pk0036.c10
w11	Wheat (<i>Triticum aestivum</i>) leaf 7 day old seedling, light grown	w11.pk0009.c9
w11n	Wheat (<i>Triticum aestivum</i>) leaf 7 day old seedling, light grown*	w11n.pk0044.b1
wr1	Wheat (<i>Triticum aestivum</i>) root; 7 day old seedling, light grown	wr1.pk0160.d11

Library	Tissue	Clone
wre1n	Wheat (<i>Triticum aestivum</i>) root; 7 day old etiolated seedling*	wre1n.pk0043.f9
		wre1n.pk0043.h8
		wre1n.pk0131.g10

*These libraries were normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

5 **V3, V4 and V9 refer to stages of corn growth. The descriptions can be found in "How a Corn Plant Develops" Special Report No. 48, Iowa State University of Science and Technology Cooperative Extension Service Ames, Iowa, Reprinted February 1993.

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science* 252:1651). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

EXAMPLE 2

25 Identification of cDNA Clones

cDNA clones encoding cellulose synthase enzymes were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The

DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nature Genetics* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

EXAMPLE 3

10 Characterization of cDNA Clones Encoding Cellulose Synthase

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to cellulose synthase from *Arabidopsis thaliana* (NCBI Identifier No. gi 2827139, gi 2827141, gi 4467125, gi 4886756 and gi 3135611) and *Gossypium hirsutum* (NCBI Identifier No. gi 1706958 and 5081779). Shown in Table 3 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), complete gene sequences ("CGS") or contigs assembled from two or more ESTs ("Contig"):

TABLE 3

20 **BLAST Results for Sequences Encoding Polypeptides Homologous to *Arabidopsis thaliana* and *Gossypium hirsutum* Cellulose Synthase**

Clone	Status	BLAST pLog Score
bsh1.pk0002.f6	FIS	154.00 (gi 2827139)
Contig composed of: cco1n.pk0005.g3 cdt2c.pk002.g1 cdt2c.pk002.l16 csc1c.pk002.i1 p0031.ccmr05rb p0110.cgsma57r	Contig	>254.00 (gi 2827141)
cr1n.pk0135.e10	FIS	176.00 (gi 1706958)
p0097.cqrad17rc	CGS	>254.00 (gi 2827141)
p0122.ckamh70rc	CGS	>254.00 (gi 2827141)
rlr24.pk0073.g1	EST	77.70 (gi 4467125)
sdp2c.pk005.o22	FIS	>254.00 (gi 4886756)
ses8w.pk0028.f3	EST	>254.00 (gi 2827139)
ssl.pk0036.c10	EST	>254.00 (gi 2827141)
Contig composed of: wl1.pk0009.c9 wr1.pk0160.d11 wre1n.pk0043.f9	Contig	>254.00 (gi 5081779)

wreln.pk0043.h8		
wreln.pk0131.g10		
wlln.pk0044.b1	EST	166.00 (gi 3135611)

Figure 1 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:2, 4, 8, 10, 12, 14, 16, 18, 20 and 22 and the *Arabidopsis thaliana* (SEQ ID NOs:23 (gi 2827139), 24 (gi 2827141), 26 (gi 4467125), 27 (gi 4886756) and 29 (gi 3135611)) and *Gossypium hirsutum* (SEQ ID NOs:25 (gi 1706958) and 28 (gi 5081779)) sequences. The data in Table 4 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 8, 10, 12, 14, 16, 18, 20 and 22 and the *Arabidopsis thaliana* (SEQ ID NOs:23, 24, 26, 27 and 29) and *Gossypium hirsutum* (SEQ ID NOs:25 and 28) sequences.

TABLE 4

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to *Arabidopsis thaliana* and *Gossypium hirsutum* Cellulose Synthase

SEQ ID NO.	Percent Identity to
2	82% (gi 2827139)
4	69% (gi 2827141)
6	89% (gi 1706958)
8	70% (gi 2827141)
10	70% (gi 2827141)
12	36% (gi 4467125)
14	86% (gi 4886756)
16	88% (gi 2827139)
18	86% (gi 2827141)
20	87% (gi 5081779)
22	70% (gi 3135611)

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones

encode a substantial portion of a cellulose synthase. These sequences represent the first barley, corn, rice, soybean and wheat sequences encoding cellulose synthase.

EXAMPLE 4

Expression of Chimeric Genes in Monocot Cells

5 A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites
10 (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the
15 plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb Sall-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-Sall fragment from the 3' end of the maize 10 kD zein gene in the vector
20 pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit;
25 U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses
30 derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic
35 proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers
5 resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used
10 to transfer genes to the callus culture cells. According to this method, gold particles (1 μm in diameter) are coated with DNA using the following technique. Ten μg of plasmid DNAs are added to 50 μL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μL of a 2.5 M solution) and spermidine free base (20 μL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After
15 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μL of ethanol. An aliquot (5 μL) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The
20 particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of
25 about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

30 Seven days after bombardment the tissue can be transferred to N6 medium that contains glufosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the
35 selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the

tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* 8:833-839).

EXAMPLE 5

Expression of Chimeric Genes in Dicot Cells

5 A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about
10 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction
15 (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

20 Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and
25 placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule.
30 Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70, U.S. Patent No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used
35 for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225

(from *E. coli*; Gritz et al.(1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then
5 be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 μ L of a 60 mg/mL 1 μ m gold particle suspension is added (in order): 5 μ L DNA (1 μ g/ μ L), 20 μ l spermidine (0.1 M), and 50 μ L CaCl₂ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 μ L 70%
10 ethanol and resuspended in 40 μ L of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five μ L of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette.
15 For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into
20 individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.
25

EXAMPLE 6

Expression of Chimeric Genes in Microbial Cells

30

The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in
35 pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using

oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

5 Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 µg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 µL of water. 10 Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent 15 cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the instant polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct 20 orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by 25 centrifugation and re-suspended in 50 µL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One µg of protein from the soluble fraction of the culture can be separated by 30 SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

EXAMPLE 7

Evaluating Compounds for Their Ability to Inhibit the Activity of Cellulose Synthase

35 The polypeptides described herein may be produced using any number of methods known to those skilled in the art. Such methods include, but are not limited to, expression in bacteria as described in Example 6, or expression in eukaryotic cell culture, *in planta*, and using viral expression systems in suitably infected organisms or cell lines. The instant

polypeptides may be expressed either as mature forms of the proteins as observed *in vivo* or as fusion proteins by covalent attachment to a variety of enzymes, proteins or affinity tags. Common fusion protein partners include glutathione S-transferase ("GST"), thioredoxin ("Trx"), maltose binding protein, and C- and/or N-terminal hexahistidine polypeptide ("His)₆"). The fusion proteins may be engineered with a protease recognition site at the fusion point so that fusion partners can be separated by protease digestion to yield intact mature enzyme. Examples of such proteases include thrombin, enterokinase and factor Xa. However, any protease can be used which specifically cleaves the peptide connecting the fusion protein and the enzyme.

Purification of the instant polypeptides, if desired, may utilize any number of separation technologies familiar to those skilled in the art of protein purification. Examples of such methods include, but are not limited to, homogenization, filtration, centrifugation, heat denaturation, ammonium sulfate precipitation, desalting, pH precipitation, ion exchange chromatography, hydrophobic interaction chromatography and affinity chromatography, wherein the affinity ligand represents a substrate, substrate analog or inhibitor. When the instant polypeptides are expressed as fusion proteins, the purification protocol may include the use of an affinity resin which is specific for the fusion protein tag attached to the expressed enzyme or an affinity resin containing ligands which are specific for the enzyme. For example, the instant polypeptides may be expressed as a fusion protein coupled to the C-terminus of thioredoxin. In addition, a (His)₆ peptide may be engineered into the N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be accomplished using other reagents which interact to displace the thioredoxin from the resin. These reagents include β-mercaptoethanol or other reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the enzyme may be accomplished after the fusion protein is purified or while the protein is still bound to the ThioBond™ affinity resin or other resin.

Crude, partially purified or purified enzyme, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit enzymatic activation of the instant polypeptides disclosed herein. Assays may be conducted under well known experimental conditions which permit optimal enzymatic activity. For example, assays for cellulose synthase activity are presented in WO 98/18949 and WO 98/00549.

CLAIMS

What is claimed is:

1. An isolated nucleic acid fragment comprising at least 900 nucleotides, wherein the nucleic acid fragment encodes a cellulose synthase comprising a member selected from the group consisting of:
 - (a) an isolated nucleic acid fragment encoding an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, 6, 12, 14, 16, 18, 20 and 22;
 - (b) an isolated nucleic acid fragment that is complementary to (a).
2. The isolated nucleic acid fragment of Claim 1 wherein nucleic acid fragment is a functional RNA.
3. The isolated nucleic acid fragment of Claim 1 wherein the nucleotide sequence of the fragment comprises the sequence set forth in a member selected from the group consisting of SEQ ID NO:1, 5, 11, 13, 15, 17, 19 and 21.
4. A chimeric gene comprising the nucleic acid fragment of Claim 1 operably linked to suitable regulatory sequences.
5. A transformed host cell comprising the chimeric gene of Claim 4.
6. A cellulose synthase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, 6, 12, 14, 16, 18, 20 and 22.
7. An isolated nucleic acid fragment encoding a cellulose synthase comprising a member selected from the group consisting of:
 - (a) an isolated nucleic acid fragment encoding an amino acid sequence that is functionally active polypeptide and at least 80% identical to the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:4, 8 and 10 ;
 - (b) an isolated nucleic acid fragment that is complementary to (a).
8. The isolated nucleic acid fragment of Claim 7 wherein nucleic acid fragment is a functional RNA.
9. The isolated nucleic acid fragment of Claim 7 wherein the nucleotide sequence of the fragment comprises the sequence set forth in a member selected from the group consisting of SEQ ID NO:3, 7 and 9.
10. A chimeric gene comprising the nucleic acid fragment of Claim 7 operably linked to suitable regulatory sequences.
11. A transformed host cell comprising the chimeric gene of Claim 10.

12. A cellulose synthase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:4, 8, 10.

5 13. A method of altering the level of expression of a cellulose synthase in a host cell comprising:

(a) transforming a host cell with the chimeric gene of any of Claims 4 and 10; and

(b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene

10 wherein expression of the chimeric gene results in production of altered levels of a cellulose synthase in the transformed host cell.

14. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a cellulose synthase comprising:

15 (a) probing a cDNA or genomic library with the nucleic acid fragment of any of Claims 1 and 7;

(b) identifying a DNA clone that hybridizes with the nucleic acid fragment any of of Claims 1 and 7;

(c) isolating the DNA clone identified in step (b); and

20 (d) sequencing the cDNA or genomic fragment that comprises the clone isolated in step (c)

wherein the sequenced nucleic acid fragment encodes all or a substantial portion of the amino acid sequence encoding a cellulose synthase.

15. A method of obtaining a nucleic acid fragment encoding a substantial portion of an amino acid sequence encoding a cellulose synthase comprising:

25 (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence set forth in any of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21; and

30 (b) amplifying a cDNA insert present in a cloning vector using the oligonucleotide primer of step (a) and a primer representing sequences of the cloning vector

wherein the amplified nucleic acid fragment encodes a substantial portion of an amino acid sequence encoding a cellulose synthase.

16. The product of the method of Claim 14.

17. The product of the method of Claim 15.

35 18. A method for evaluating at least one compound for its ability to inhibit the activity of a cellulose synthase, the method comprising the steps of:

- 5
- 10
- (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a cellulose synthase, operably linked to suitable regulatory sequences;
 - (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the cellulose synthase encoded by the operably linked nucleic acid fragment in the transformed host cell;
 - (c) optionally purifying the cellulose synthase expressed by the transformed host cell;
 - (d) treating the cellulose synthase with a compound to be tested; and
 - (e) comparing the activity of the cellulose synthase that has been treated with a test compound to the activity of an untreated cellulose synthase, thereby selecting compounds with potential for inhibitory activity.

Figure 1

```

1
SEQ ID NO: 2 ----- 60
SEQ ID NO: 4 RAAQAQRNKGKQPPEEQKLASVSLP--LPHSRFIPFPPRRRYRRR---RTHACPG----I
SEQ ID NO: 6 -----
SEQ ID NO: 8 HSSYTKSRSSLAQPRAAPRQAQPPP--ATAACACERSPRPGDQRRGGLRAFRCAAAGFV
SEQ ID NO: 10 -----
SEQ ID NO: 12 RCS---RRWTCSSPPPTPTRSRSPRTP-----
SEQ ID NO: 14 -----
SEQ ID NO: 16 -----
SEQ ID NO: 18 -----
SEQ ID NO: 20 -----
SEQ ID NO: 22 -----
SEQ ID NO: 23 -----
SEQ ID NO: 24 MNTGGR-----
SEQ ID NO: 25 -----
SEQ ID NO: 26 MASTPPQTSKKVRNNSGSGQTVKFARRTSSGRYVSLR-RDNIELSGELSGDYSNYTVHIP
SEQ ID NO: 27 -----
SEQ ID NO: 28 -----
SEQ ID NO: 29 R---PR-----

61
SEQ ID NO: 2 ----- 120
SEQ ID NO: 4 W-RSGSARG----ME-ASAGLVAGSHNRNELV-VIRRDGEPGPKP--MDQRNGQVCQI--
SEQ ID NO: 6 -----
SEQ ID NO: 8 RERDPAGRGGGPEME-ASAGLVAGSHNRNELV-VIRRDRESGAAGGAARRAEPQCI--
SEQ ID NO: 10 -----ME-ASAGLVAGSHNRNELV-VIRRDGDPGPKP--PREQNGQVCQI--
SEQ ID NO: 12 -----C-----
SEQ ID NO: 14 -----ME-ASAGLVAGSHNRNELV-VIHGHEEP--KA--LKNLDGQVCEI--
SEQ ID NO: 16 -----
SEQ ID NO: 18 -----
SEQ ID NO: 20 -----
SEQ ID NO: 22 -----
SEQ ID NO: 23 -----ME-ASAGLVAGSYRRNELV-RIRHESDGGTKP--LKNMNGQICQI--
SEQ ID NO: 24 -----LIAGSHNRNEFV-LI--NADESARIRSVQELSGQTCQI--
SEQ ID NO: 25 -----
SEQ ID NO: 26 PTPDNQPMATKAEEQYVSNLFTGGFNSVTRAHLMDKVIDSDVTHPQAGAKGSSCAMP
SEQ ID NO: 27 -----ME-ASAGLVAGSHNRNELV-VIHNHEEP--KP--LKNLDGQFCEI--
SEQ ID NO: 28 -----
SEQ ID NO: 29 -----LIAGSHNRNEFV-LI--NADENARIRSVQELSGQTCQI--

121
SEQ ID NO: 2 ----- 180
SEQ ID NO: 4 CGDDVGRNPDGEPFVACNECAFPICRDCYERREGTQNCPOCKTRFKRLKGCARVPGD-
SEQ ID NO: 6 -----
SEQ ID NO: 8 CGDEVGVGFDGEPFVACNECAFVCRACYEYERREGSQACPOCRTRYKRLKGCPRVAGD-
SEQ ID NO: 10 CGDDVGLAPGGDPFVACNECAFVCRDCYERREGTQNCPOCKTRYKRLKGCQRVTDG-
SEQ ID NO: 12 -----CPY-----
SEQ ID NO: 14 CGDGVGLTVDGDLFVACNECGFPVCRPCYERREGSHLCPQCKTRYKRLKGSPRVEGDD
SEQ ID NO: 16 -----
SEQ ID NO: 18 -----
SEQ ID NO: 20 -----
SEQ ID NO: 22 -----
SEQ ID NO: 23 CGDDVGLAETGDVAVACNECAFVCRPCYERKDGTCQCPQCKTRFRHRGSPRVEGDE
SEQ ID NO: 24 CGDEIELTVSSELFVACNECAFVCRPCYERREGNQACPOCKTRYKRIKGSPRVDGDD
SEQ ID NO: 25 -----
SEQ ID NO: 26 CDGNVMDKDERGKDVMPG-ECRFKICRDCFMDAQE-TGLCPGCKEQYK-----

```

Figure 1 (cont'd.)

SEQ ID NO:27 CGDQIGLTVEGDLFVACNECGFPACRPCYEYERREGTQNCPCQCKTRYKRLRGSPRVEGDE
 SEQ ID NO:28 -----
 SEQ ID NO:29 CRDEIELTVDGEPFVACNECAFVCRPCYEYERREGNQACPQCKTRFKRLKGSPRVEGD-

181

240

SEQ ID NO:2 -----
 SEQ ID NO:4 EEEDGVDDLENEFNWSDK----HDSQYLAESMLHAHMSYG-RGADLDGVPQPFHPIPNVP
 SEQ ID NO:6 -----
 SEQ ID NO:8 EEEDGVDDLEGEFGLQDGAHEDDPQYVAESMLRAQMSYG-RGGDAH---PGFSPVPNVP
 SEQ ID NO:10 EEEDGVDDLNEFNW-DG----HDSQVAESMLYGHMSYG-RGGDPNGAPQAFQLNPNVP
 SEQ ID NO:12 -----
 SEQ ID NO:14 DEEDV-DDIEHEFNIDEQKNKHGQ---VAEAMLHGRMSYG--RGPEDDNSQFPTPVIAG
 SEQ ID NO:16 -----
 SEQ ID NO:18 -----
 SEQ ID NO:20 -----
 SEQ ID NO:22 -----
 SEQ ID NO:23 DEDDV-DDIENEFNYAQANKARH---QRHGE---EFSSS--SRHESQIPLLTHGHTVS
 SEQ ID NO:24 EEEEDIDDLEYEFD-----HGMDPEHAEEAALSSRLNTG--RGGLD SAPPG-----SQIP
 SEQ ID NO:25 -----
 SEQ ID NO:26 -----IGDLDD-----TPDYSSGALPLPAPG-----
 SEQ ID NO:27 DEEDI-DDIEYEFNIEHEQDKKH---SAEAMLYGKMSYG--RGPEDDENGRFP-PVIAG
 SEQ ID NO:28 -----
 SEQ ID NO:29 EEEDDIDDLNEFEYGN---NGIGFDQVSEGMSISRRNSGFQSDLDSAPPG-----SQIP

241

300

SEQ ID NO:2 -----
 SEQ ID NO:4 LLTNGQMVDIPPDQHALVPSFV---GGGKRIHPLPYADPNLPVQPRSM DPSKDLAAYG
 SEQ ID NO:6 -----
 SEQ ID NO:8 LLTNGQMVDIPPEQHALVPSYMSGGGGGGKRIHPLPFADPNLPVQPRSM DPSKDLAAYG
 SEQ ID NO:10 LLTNGQMVDIPPEQHALVPSFM---GGGKRIHPLPYADPSLPVQPRSM DPSKDLAAYG
 SEQ ID NO:12 -----
 SEQ ID NO:14 GRSR-----PVSGEFPISSNAYGDQMLSSSLHKRVHPYPVSEPGSARW-----DEKXKDG
 SEQ ID NO:16 -----
 SEQ ID NO:18 -----
 SEQ ID NO:20 -----
 SEQ ID NO:22 LLTNGQMVDIPPEQHALVPSYMSGGGGGGKRIHPLPFADPNLPVQPRSM DPSKDLAAYG
 SEQ ID NO:23 GEIRTPDTQSVRTTSGPLGPSDRNAISSPYIDPR-QPVPVRIVDPSK-----DLNSYG
 SEQ ID NO:24 LLTYCDEDADMYSDRHALIVP--PS-TGYGNRVYPAPFTDSSAPPQARS MVPQKDIAEYG
 SEQ ID NO:25 -----
 SEQ ID NO:26 -----KDQRGNNNMMSMKRNQNGEFDHNRWLF-----ETQGTYG
 SEQ ID NO:27 GHS-----GEFPVGG-GYGNG--EHGLHKRVHPYPSSEAGS-----EGG
 SEQ ID NO:28 -----
 SEQ ID NO:29 LLTYGDEDVEISSDRHALIVP--PSLGGHGNRVHPVLSLSDPTVAHRRLMVPQKDLAVYG

301

360

SEQ ID NO:2 -----
 SEQ ID NO:4 YGSVAWKERMESWKQKQ-ERMHQTRNDGGGD-----DGDDADLPLM-DEARQPLSR
 SEQ ID NO:6 -----
 SEQ ID NO:8 YGSVAWKERMEGWKQKQ-ERLQHVRESEGGGDW-----DGDDADLPLM-DEARQPLSR
 SEQ ID NO:10 YGSVAWKERMENWKQRQ-ERMHQTGNDGGGD-----DGDDADLPLM-DEARQQLSR
 SEQ ID NO:12 -----
 SEQ ID NO:14 -----WKDRMDDWKLQQG-----NLGPEPDEDPAAML-DEARQPLSR
 SEQ ID NO:16 -----
 SEQ ID NO:18 -----HE-----
 SEQ ID NO:20 -----
 SEQ ID NO:22 YGSVAWKERMEGWKQKQ-ERLQHVRESEGGGDW-----DGDDADLPLM-DEARQPLSR
 SEQ ID NO:23 LGNVDWKERVEGWKLKQEKNLQMTGKYHEGKGG-EIEGTGSNGEELQM-ADDTRLPMSR

Figure 1 (cont'd.)

SEQ ID NO:24 YGSVAWKDRMEVWKRROGEKLVKHEGGNNGRGSN--DDELDDPDMPM--DEGRQPLSR
 SEQ ID NO:25 -----
 SEQ ID NO:26 YGNAYWP-----QDEMYGD-----DMDEGMRGGMVETADKPWRPLSR
 SEQ ID NO:27 -----WRERMDDWKLOHG-----NLGPEPDDDPENGLI--DEARQPLSR
 SEQ ID NO:28 -----
 SEQ ID NO:29 YGSVAWKDRMEEWKRKQNEKLQVVRHEGDP-----DFEDGDDADFMM--DEGRQPLSM

361

420

SEQ ID NO:2 -----
 SEQ ID NO:4 KIPLPSSQINPYRMIIRLVLVLCFFFHYRVMHPVDAFALWLISVCEIWFAMSWILDQ
 SEQ ID NO:6 -----
 SEQ ID NO:8 KVPISSSRINPYRMIIVIRLVVLGFFFHYRVMHPAKDAFALWLISVCEIWFAMSWILDQ
 SEQ ID NO:10 KIPLPSSQINPYRMIIRLVLVLCFFFHYRVMHPVNDAFALWLISVCEIWFAMSWILDQ
 SEQ ID NO:12 -----
 SEQ ID NO:14 KVPIASSKINPYRMVIVARLVILAFFLRYRLMNPVHDALGLWLTSIICEIWFAFSWILDQ
 SEQ ID NO:16 -----
 SEQ ID NO:18 -----LHPVNDAYGLWLTSVCEIWFVAVSWIMDQ
 SEQ ID NO:20 -----
 SEQ ID NO:22 KVPISSSRINPYRMIIVIRLVVLGFFFHYRVMHPAKDAFALWLISVCEIWFAMSWILDQ
 SEQ ID NO:23 VVIPSSRLTPYRVVILRLIILCFFLQYRTHPVKNAYPLWLTSVCEIWFVAVSWILDQ
 SEQ ID NO:24 KLPISSSRINPYRMLILCRLAILGLFFHYRILHPVNDAYGLWLTSVCEIWFVAVSWILDQ
 SEQ ID NO:25 -----
 SEQ ID NO:26 RIPIPAAIISPYRLLIVIRFVVLCTWRIRNPNEDAIWLWMSIICELWFGFSWILDQ
 SEQ ID NO:27 KVPIASSKINPYRMVIVARLVILAVFLRYRLMNPVHDALGLWLTSVCEIWFVAVSWILDQ
 SEQ ID NO:28 -----
 SEQ ID NO:29 KIPIKSSKINPYRMLIVLRLVILGLFFHYRILHPVKDAYALWLISVCEIWFVAVSWILDQ

421

480

SEQ ID NO:2 -----
 SEQ ID NO:4 FPKWFPIERETYLDRSLRFDKEGHPS-----QLAPVDFVSTVDPLKEPPLVTANTVLS
 SEQ ID NO:6 -----
 SEQ ID NO:8 FPKWLPPIERETYLDRSLRFDKEGQPS-----QLAPIDFFVSTVDPTKEPPLVTANTVLS
 SEQ ID NO:10 FPKWFPIERETYLDRSLRFDKEGQPS-----QLAPIDFFVSTVDPLKEPPLVTANTVLS
 SEQ ID NO:12 -----
 SEQ ID NO:14 FPKWFPIERETYLDRSLRFDKEGQPS-----MLAPVDVVFVSTVDPMPKEPPLVTANTVLS
 SEQ ID NO:16 -----
 SEQ ID NO:18 FPKWYPIQRETYLDRSLRYEKEGKPS-----ELSSVDVVFVSTVDPMPKEPPLITANTVLS
 SEQ ID NO:20 -----
 SEQ ID NO:22 FPKWFPIERETYLDRSLRFDKEGQPS-----QLAPIDFFVSTVDPTKEPPLVTANTVLS
 SEQ ID NO:23 FPKWYPINRETYLDRSLRYEKEGQPS-----QLVPVDVVFVSTVDPLKEPPLVTANTVLS
 SEQ ID NO:24 FPKWYPIERETYLDRSLRYEKEGKPS-----GLAPVDVVFVSTVDPLKEPPLITANTVLS
 SEQ ID NO:25 -----
 SEQ ID NO:26 IPKLCPINRSTDLEVLDRKFDMPSPSNPTGRSDLPGIDLDFVSTADPEKEPPLVTANTVLS
 SEQ ID NO:27 FPKWFPIERETYLDRSLRYEKEGQPS-----MLAPVDVVFVSTVDPLKEPPLVTANTVLS
 SEQ ID NO:28 -----
 SEQ ID NO:29 FPKWYPIERETYLDRSLRYEKEGKPS-----GLSPVDVVFVSTVDPLKEPPLITANTVLS

481

540

SEQ ID NO:2 -----
 SEQ ID NO:4 ILSVDYPVDKVCYVSDGAAMLTFEALSETSEFAKKWVPFCKRYSLEPRAPEWYFQO--
 SEQ ID NO:6 -----H-----
 SEQ ID NO:8 ILSVDYPVEKVCYVSDGAAMLTFEALSETSEFAKKWVPFCKRYSLEPRAPEWYFQO--
 SEQ ID NO:10 ILSVDYPVDKVCYVSDGAAMLTFEALSETSEFAKKWVPFCKRYSLEPRAPEWYFQO--
 SEQ ID NO:12 ILAAGYPAGKVTCTYISDDAGAEVTRNAVVEARFAALWVFCRKHGVEPRNLEAYFNAGE
 SEQ ID NO:14 ILAMDYPVDKVCYVSDGASMTFESLSETAEFARKWVPFCKRYSLEPRAPEWYFQO--
 SEQ ID NO:16 -----
 SEQ ID NO:18 ILAVDYPVDKVCYVSDGAAMLTFEALSETSEFARRWVPFCKRYSLEPRAPEWYFQO--

Figure 1 (cont'd.)

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SEQ ID NO:20 -----
SEQ ID NO:22 ILSVDYPVEKVSICYVSDDGAAMLTFEALSETSEFAKKWVPFSKKNIEPRAPEWYFQQ--
SEQ ID NO:23 ILSVDYPVDKVVACYVSDDGSAMLTFESLSETAEFAKKWVPFCKKNIEPRAPEFYFAQ--
SEQ ID NO:24 ILAVDYPVDKVVACYVSDDGAAMLTFEALSDTAEFARKWVPFCKKNIEPRAPEWYFSQ--
SEQ ID NO:25 -----RRWVPFCKKHNVPRPEFYFNE--
SEQ ID NO:26 ILAVDYPVEKVSICYLSDDGGALLSFEAMAEEASFADLWVPFCRKHNIEPNPDSYFSL--
SEQ ID NO:27 ILAMDYPVEKISICYVSDDGASMLTFESLSETAEFAKKWVPFCKKFSIEPRAPEMYFTL--
SEQ ID NO:28 ----DYPVEKVSICYVSDDGAAMLTFEALSETSEFAKKWVPFCKKYNIEPRAPEWYFAQ--
SEQ ID NO:29 ILAVDYPVDKVVACYVSDDGAAMLTFEALSETAEFAKKWVPFCKKYCIEPRAPEWYFCH--

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541

600

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SEQ ID NO:2 -----
SEQ ID NO:4 -----KIDYLDKDKVAPNFVRERRAMKREYEEFKVRINALVAKAQ-----
SEQ ID NO:6 -----
SEQ ID NO:8 -----KIDYLDKDKVAASFVRERRAMKREYEEFKVRINALVAKAQ-----
SEQ ID NO:10 -----KIDYLDKDKVAANFVRERRAMKREYEEFKVRINALVAKAQ-----
SEQ ID NO:12 GGGGKAKVVARGSY-RGMAWPELVRDRRRVREYEEMRLRIDALQAADARRR-----
SEQ ID NO:14 -----KIDYLDKDKVQPTFVKERRAMKREYEEFKVRINALVAKAQ-----
SEQ ID NO:16 -----AKAQ-----
SEQ ID NO:18 -----KMDYLKNKVHPAFVRERRAMKRDYEEFKVRINSLVATAQ-----
SEQ ID NO:20 -----
SEQ ID NO:22 -----KIDYLDKDKVAASFVRERRAMKREYEEFKVRINALVAKAQ-----
SEQ ID NO:23 -----KIDYLDKDKIQPSFVKERRAMKREYEEFKVRINALVAKAQ-----
SEQ ID NO:24 -----KMDYLKNKVHPAFVRERRAMKRDYEEFKVKINALVATAQ-----
SEQ ID NO:25 -----KIDYLDKDKVHPSFVKERRAMKREYEEFKVRINALVAKAQ-----
SEQ ID NO:26 -----KIDPTKNKSRIDFVKDRRKIKREYDEFKVRINGLPDSIRRRSDAFNAREE-----
SEQ ID NO:27 -----KVDYLDQDKVHPTFVKERRAMKREYEEFKVRINAQVAKAS-----
SEQ ID NO:28 -----KIDYLDKDKVQTSFVKERRAMKREYEEFKVRVNLVAKAQ-----
SEQ ID NO:29 -----KMDYLKNKVHPAFVRERRAMKRDYEEFKVKINALVATAQ-----

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601

660

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SEQ ID NO:2 -----
SEQ ID NO:4 -----KVPEEGWTMQDGTWPWG-----NNVRDHPGMIQVFL---
SEQ ID NO:6 -----
SEQ ID NO:8 -----KVPEEGWTMQDGSWPWG-----NNVRDHPGMIQVFL---
SEQ ID NO:10 -----KVPEEGWTMQDGTWPWG-----NNVRDHPGMIQVFL---
SEQ ID NO:12 -----RRGAADDHAGVVQVLIDFA-----
SEQ ID NO:14 -----KVPOGGWIMQDGTWPWG-----NNTKDHPGMIQVFL---
SEQ ID NO:16 -----KMPEEGWTMQDGTWPWG-----NNPRDHPGMIQVFL---
SEQ ID NO:18 -----KVPEDGWTMQDGTWPWG-----NNVRDHPGMIQVFL---
SEQ ID NO:20 -----
SEQ ID NO:22 -----KVPEEGWTMQDGSWPWG-----
SEQ ID NO:23 -----KIPEEGWTMQDGTWPWG-----NNTRDHPGMIQVFL---
SEQ ID NO:24 -----KVPEEGWTMQDGTWPWG-----NNVRDHPGMIQVFL---
SEQ ID NO:25 -----KKPEEGWVMQDGTWPWG-----NNTRDHPGMIQVYL---
SEQ ID NO:26 MKALKQMRESGGDPTFPVKPKATW-MADGTHWPGTWAASTREHSKGDHAGILQVMLKPP
SEQ ID NO:27 -----KVPLEGWIMQDGTWPWG-----NNTKDHPGMIQVFL---
SEQ ID NO:28 -----KVPEEGWIMQDGTWPWG-----NNTRDHPGMIQVFL---
SEQ ID NO:29 -----KVPEDGWTMQDGTWPWG-----NSVRDHPGMIQVFL---

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661

720

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SEQ ID NO:2 -----
SEQ ID NO:4 -----G-QSGGHDVE----GNELPRLVYVSREKRPYGNHKKAGAMNALVRVSAVLTNA
SEQ ID NO:6 -----
SEQ ID NO:8 -----G-QSGGRDVE----GNELPRLVYVSREKRPYGNHKKAGAMNALVRVSAVLSNA
SEQ ID NO:10 -----G-QSGGLDCE----GNELPRLVYVSREKRPYGNHKKAGAMNALVRVSAVLTNA
SEQ ID NO:12 GSVLPQLGVANGSKLIDVASVDVCLPALVYVCREKRRGHAHHRKAGAMNA-----

```

Figure 1 (cont'd.)

SEQ ID NO:14 -----G-SSGGLDTE----GNQLPRLVYVSREKRPGFQHHKKAGAMNALVRVSAVLTNA
 SEQ ID NO:16 -----G-HSGGLDTD----GNELPRLVYVSREKRPGFQHHKKAGAMNALIRVSAVLTNG
 SEQ ID NO:18 -----G-QDGV RDVE----GNELPRLVYVSREKRPGFQHHKKAGAMNALVRASAIITNA
 SEQ ID NO:20 -----
 SEQ ID NO:22 -----
 SEQ ID NO:23 -----G-HSGGLDTD----GNELPRLIYVSREKRPGFQHHKKAGAMNALIRVSAVLTNG
 SEQ ID NO:24 -----G-HSGV RDTD----GNELPRLVYVSREKRPGFQHHKKAGAMNSLIRVSAVLSNA
 SEQ ID NO:25 -----G-SAGALDVD----GKELPRLVYVSREKRPQYQHHKKAGAENALVRVSAVLTNA
 SEQ ID NO:26 SSDPLIG-NSDDKVIDFSDTDTRLPMFVYVSREKRPQYDHNKKAGAMNALVRASAILSNG
 SEQ ID NO:27 -----G-HSGGFDVE----GHLPRLVYVSREKRPGFQHHKKAGAMNALVRVAGVLTNA
 SEQ ID NO:28 -----G-QSGGLDAE----GNELPRLVYVSREKRPGFQHHKKAGAMNALVRVSAVLTNG
 SEQ ID NO:29 -----G-SDGV RDVE----NNELPRLVYVSREKRPGFQHHKKAGAMNSLIRVSGVLSNA

721

780

SEQ ID NO:2 -----
 SEQ ID NO:4 PYLLNLDCDHYINNSKAIKEAMCFMMDPLLGGK-----VCYVQFPQRFDGIDRHDRYAN
 SEQ ID NO:6 -----
 SEQ ID NO:8 AYLLNLDCDHYINNSKAIKEAMCFMMDPLVGGK-----VCYVQFPQRFDGIDKNDRYAN
 SEQ ID NO:10 PYLLNLDCDHYINNSKAIKEAMCFMMDPLLGGK-----VCYVQFPQRFDGIDRHDRYAN
 SEQ ID NO:12 PFILDLDYVYVNSQALRAGICFMIERGGGAAEDAGAVAFVQFPQRFVGDVDPGDRYAN
 SEQ ID NO:14 PFMLNLDCDHYVNSKAAREAMCFMLDPQTGGK-----VCYVQFPQRFDGIDRHDRYAN
 SEQ ID NO:16 AYLLNVDCDHYFNNSKALKEAMCFMMDPVLGGK-----TCYVQFPQRFDGIDLHDRYAN
 SEQ ID NO:18 PYLLNVDCDHYINNSKALREAMCFMMDPQLGGK-----VCYVQFPQRFDGIDRHDRYSN
 SEQ ID NO:20 -----EAMCFMLDPNLGPQ-----VCYVQFPQRFDGIDRNDRYAN
 SEQ ID NO:22 -----
 SEQ ID NO:23 AYLLNVDCDHYFNNSKAIKEAMCFMMDPAIGKK-----CCYVQFPQRFDGIDLHDRYAN
 SEQ ID NO:24 PYLLNVDCDHYINNSKAIRESMCFMMDPQSGKK-----VCYVQFPQRFDGIDRHDRYSN
 SEQ ID NO:25 PFILNLDCDHYINNSKAMREAMCFMLDPQFGKK-----LCYVQFPQRFDGIDRHDRYAN
 SEQ ID NO:26 PFILNLDCDHYIYNCKAVREGMCFMMDRG-GED-----ICYIQFPQRFEGIDPSDRYAN
 SEQ ID NO:27 PFMLNLDCDHYVNSKAAREAMCFMLDPQIGKK-----VCYVQFPQRFDGIDTNDRYAN
 SEQ ID NO:28 AFLNLDCDHYINNSKALREAMCFMLDPNLGGKQ-----VCYVQFPQRFDGIDRNDRYAN
 SEQ ID NO:29 PYLLNVDCDHYINNSKALREAMCFMMDPQSGKK-----ICYVQFPQRFDGIDRHDRYSN

781

840

SEQ ID NO:2 -----
 SEQ ID NO:4 RNVVFFDINMKGLDGIQGPVYVGTGCVFRRQALYGYDAP---KTKKPPSRTCNCWPKWCI
 SEQ ID NO:6 -----
 SEQ ID NO:8 RNVVFFDINMKGLDGIQGPVYVGTGCVFRRQALYGYDAP---KTKKPPSRTCNCWPKWCL
 SEQ ID NO:10 RNVVFFDINMKGLDGIQGPVYVGTGCVFRRQALYGYDAP---KTKKPPSRTCNCWPKWCF
 SEQ ID NO:12 HNRVLFDC TELGLDGLQGPVYVGTGCLFRRVALYSVDLPR-----
 SEQ ID NO:14 RNTVFFDINMKGLDGIQGPVYVGTGCVFRRQALYGYNPPKGPK--RPKMVS CDC-----
 SEQ ID NO:16 RNIVFFDINMKGQDGVQGPVYVGTGCCFNROALYGYDPVLTEEDLE-----PNIIV
 SEQ ID NO:18 RNVVFFDINMKGLDGIQGPVYVGTGCVFRRYALYGYDAP---AKKKPPSKTCNCWPKWCC
 SEQ ID NO:20 RNTVFFDINLRGLDGIQGPVYVGTGCVFNRTAIYGYEPPKAK--K-----PGFLA
 SEQ ID NO:22 -----
 SEQ ID NO:23 RNIVFFDINMKGLDGIQGPVYVGTGCCFNROALYGYDPVLTEEDLE-----PNIIV
 SEQ ID NO:24 RNVVFFDINMKGLDGIQGPVYVGTGCVFRRQALYGF DAP---KTKKPPGKTCNCWPKWCC
 SEQ ID NO:25 RNVVFFDINMLGLDGLQGPVYVGTGCVFNROALYGYDPPVSEKRPK---MTCDCWPSWCC
 SEQ ID NO:26 NNTVFFDGNMRALDGVQGPVYVGTGTMFRRFALYGFDP-----
 SEQ ID NO:27 RNTVFFDINMKGLDGIQGPVYVGTGCVFKRQALYGYEPPKGPK--RPKMISCGC-----
 SEQ ID NO:28 RNTVFFDINLRGLDGIQGPVYVGTGCVFNRTALYGYE PPLKPKHRK-----TGILS
 SEQ ID NO:29 RNVVFFDINMKGLDGLQGPVYVGTGCVFRRQALYGF DAP---KTKKGRKTCNCWPKWCL

Figure 1 (cont'd.)

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 <212> PRT
 <213> Zea mays

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 35 40 45
 Pro Gly Ile Trp Arg Ser Gly Ser Ala Arg Gly Met Glu Ala Ser Ala
 50 55 60
 Gly Leu Val Ala Gly Ser His Asn Arg Asn Glu Leu Val Val Ile Arg
 65 70 75 80
 Arg Asp Gly Glu Pro Gly Pro Lys Pro Met Asp Gln Arg Asn Gly Gln
 85 90 95
 Val Cys Gln Ile Cys Gly Asp Asp Val Gly Arg Asn Pro Asp Gly Glu
 100 105 110
 Pro Phe Val Ala Cys Asn Glu Cys Ala Phe Pro Ile Cys Arg Asp Cys
 115 120 125
 Tyr Glu Tyr Glu Arg Arg Glu Gly Thr Gln Asn Cys Pro Gln Cys Lys
 130 135 140
 Thr Arg Phe Lys Arg Leu Lys Gly Cys Ala Arg Val Pro Gly Asp Glu
 145 150 155 160
 Glu Glu Asp Gly Val Asp Asp Leu Glu Asn Glu Phe Asn Trp Ser Asp
 165 170 175
 Lys His Asp Ser Gln Tyr Leu Ala Glu Ser Met Leu His Ala His Met
 180 185 190
 Ser Tyr Gly Arg Gly Ala Asp Leu Asp Gly Val Pro Gln Pro Phe His
 195 200 205
 Pro Ile Pro Asn Val Pro Leu Leu Thr Asn Gly Gln Met Val Asp Asp
 210 215 220
 Ile Pro Pro Asp Gln His Ala Leu Val Pro Ser Phe Val Gly Gly Gly
 225 230 235 240
 Gly Lys Arg Ile His Pro Leu Pro Tyr Ala Asp Pro Asn Leu Pro Val
 245 250 255
 Gln Pro Arg Ser Met Asp Pro Ser Lys Asp Leu Ala Ala Tyr Gly Tyr
 260 265 270
 Gly Ser Val Ala Trp Lys Glu Arg Met Glu Ser Trp Lys Gln Lys Gln
 275 280 285

Glu Arg Met His Gln Thr Arg Asn Asp Gly Gly Gly Asp Asp Gly Asp
 290 295 300
 Asp Ala Asp Leu Pro Leu Met Asp Glu Ala Arg Gln Pro Leu Ser Arg
 305 310 315 320
 Lys Ile Pro Leu Pro Ser Ser Gln Ile Asn Pro Tyr Arg Met Ile Ile
 325 330 335
 Ile Ile Arg Leu Val Val Leu Cys Phe Phe Phe His Tyr Arg Val Met
 340 345 350
 His Pro Val Pro Asp Ala Phe Ala Leu Trp Leu Ile Ser Val Ile Cys
 355 360 365
 Glu Ile Trp Phe Ala Met Ser Trp Ile Leu Asp Gln Phe Pro Lys Trp
 370 375 380
 Phe Pro Ile Glu Arg Glu Thr Tyr Leu Asp Arg Leu Ser Leu Arg Phe
 385 390 395 400
 Asp Lys Glu Gly His Pro Ser Gln Leu Ala Pro Val Asp Phe Phe Val
 405 410 415
 Ser Thr Val Asp Pro Leu Lys Glu Pro Pro Leu Val Thr Ala Asn Thr
 420 425 430
 Val Leu Ser Ile Leu Ser Val Asp Tyr Pro Val Asp Lys Val Ser Cys
 435 440 445
 Tyr Val Ser Asp Asp Gly Ala Ala Met Leu Thr Phe Glu Ala Leu Ser
 450 455 460
 Glu Thr Ser Glu Phe Ala Lys Lys Trp Val Pro Phe Cys Lys Arg Tyr
 465 470 475 480
 Ser Leu Glu Pro Arg Ala Pro Glu Trp Tyr Phe Gln Gln Lys Ile Asp
 485 490 495
 Tyr Leu Lys Asp Lys Val Ala Pro Asn Phe Val Arg Glu Arg Arg Ala
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 Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn Ala Leu Val
 515 520 525
 Ala Lys Ala Gln Lys Val Pro Glu Glu Gly Trp Thr Met Gln Asp Gly
 530 535 540
 Thr Pro Trp Pro Gly Asn Asn Val Arg Asp His Pro Gly Met Ile Gln
 545 550 555 560
 Val Phe Leu Gly Gln Ser Gly Gly His Asp Val Glu Gly Asn Glu Leu
 565 570 575
 Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly Tyr Asn His
 580 585 590
 His Lys Lys Ala Gly Ala Met Asn Ala Leu Val Arg Val Ser Ala Val
 595 600 605

Leu Thr Asn Ala Pro Tyr Leu Leu Asn Leu Asp Cys Asp His Tyr Ile
 610 615 620
 Asn Asn Ser Lys Ala Ile Lys Glu Ala Met Cys Phe Met Met Asp Pro
 625 630 635 640
 Leu Leu Gly Lys Lys Val Cys Tyr Val Gln Phe Pro Gln Arg Phe Asp
 645 650 655
 Gly Ile Asp Arg His Asp Arg Tyr Ala Asn Arg Asn Val Val Phe Phe
 660 665 670
 Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly Pro Ile Tyr Val
 675 680 685
 Gly Thr Gly Cys Val Phe Arg Arg Gln Ala Leu Tyr Gly Tyr Asp Ala
 690 695 700
 Pro Lys Thr Lys Lys Pro Pro Ser Arg Thr Cys Asn Cys Trp Pro Lys
 705 710 715 720
 Trp Cys Ile Cys Cys Cys Cys Phe Gly Asn Arg Lys Thr Lys Lys Lys
 725 730 735
 Thr Lys Thr Ser Lys Pro Lys Phe Glu Lys Ile Lys Lys Leu Phe Lys
 740 745 750
 Lys Lys Glu Asn Gln Ala Pro Ala Tyr Ala Leu Gly Glu Ile Asp Glu
 755 760 765
 Ala Ala Pro Gly Ala Glu Asn Glu Lys Ala Ser Ile Val Asn Gln Gln
 770 775 780
 Lys Leu Glu Lys Lys Phe Gly Gln Ser Ser Val Phe Val Ala Ser Thr
 785 790 795 800
 Leu Leu Glu Asn Gly Gly Thr Leu Lys Ser Ala Ser Pro Ala Ser Leu
 805 810 815
 Leu Lys Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr
 820 825 830
 Gly Trp Gly Lys Asp Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp
 835 840 845
 Ile Leu Thr Gly Phe Lys Met His Cys His Gly Trp Arg Ser Ile Tyr
 850 855 860
 Cys Ile Pro Lys Arg Ala Ala Phe Lys Gly Ser Ala Pro Leu Asn Leu
 865 870 875 880
 Ser Asp Arg Leu His Gln Val Leu Arg Trp Ala Leu Gly Ser Ile Glu
 885 890 895
 Ile Phe Phe Ser Asn His Cys Pro Leu Trp Tyr Gly Tyr Gly Gly Gly
 900 905 910
 Leu Lys Phe Leu Glu Arg Phe Ser Tyr Ile Asn Ser Ile Val Tyr Pro
 915 920 925

Trp Thr Ser Ile Pro Leu Leu Ala Tyr Cys Thr Leu Pro Ala Ile Cys
 930 935 940

Leu Leu Thr Gly Lys Phe Ile Thr Pro Glu Leu Asn Asn Val Ala Ser
 945 950 955 960

Leu Trp Phe Met Ser Leu Phe Ile Cys Ile Phe Ala Thr Ser Ile Leu
 965 970 975

Glu Met Arg Trp Ser Gly Val Gly Ile Asp Asp Trp Trp Arg Asn Glu
 980 985 990

Gln Phe Trp Val Ile Gly Gly Val Ser Ser His Leu Phe Ala Val Phe
 995 1000 1005

Gln Gly Leu Leu Lys Val Ile Ala Gly Val Asp Thr Ser Phe Thr Val
 1010 1015 1020

Thr Ser Lys Gly Gly Asp Asp Glu Glu Phe Ser Glu Leu Tyr Thr Phe
 1025 1030 1035 1040

Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Leu Leu Leu Leu Asn
 1045 1050 1055

Phe Ile Gly Val Val Ala Gly Ile Ser Asn Ala Ile Asn Asn Gly Tyr
 1060 1065 1070

Glu Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Phe Trp Val
 1075 1080 1085

Ile Val His Leu Tyr Pro Phe Leu Lys Gly Leu Val Gly Arg Gln Asn
 1090 1095 1100

Arg Thr Pro Thr Ile Val Ile Val Trp Ser Ile Leu Leu Ala Ser Ile
 1105 1110 1115 1120

Phe Ser Leu Leu Trp Val Arg Ile Asp Pro Phe Leu Ala Lys Asp Asp
 1125 1130 1135

Gly Pro Leu Leu Glu Glu Cys Gly Leu Asp Cys Asn
 1140 1145

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 <211> 1189
 <212> DNA
 <213> Zea mays

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 gccggcgctt aaggggctcg cgcccatcaa cttgtctgat cgtctccacc aggtgctgcg 180
 ctgggcgctg gggctccgtg agatcttcat gagccgccac tgcccgctct ggtacgccta 240
 cggcggcccg ctcaagtggc tggagcgtt cgcctacacc aacaccatcg tgtaccctt 300
 cacctccatc ccgctcctcg cctactgcac catccccgcc gtctgcctgc tcaccggcaa 360
 gttcatcatt cccacgctga acaacctcgc cagcatctgg ttcategcgc tcttctgtc 420
 catcatcgcg acgagcgtcc tggagctgcg gtggagcggg gtgagcatcg aggactggtg 480
 gcgcaacgag cagttctggg tcatcggcgg cgtgtccgcy catctcttcg ccgtgttcca 540
 gggcttctc aaggttctgg gcggcgtgga caccagcttc accgtcacct ccaaggcggc 600
 cggcgacgag gccgacgcct tcggggacct ctacctctc aagtggacca ccctgctggt 660
 gccccccacc acgctcatca tcatcaacat ggtgggcatc gtggccggcg tgtccgacgc 720

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cgtaacaac ggctacggct cctggggccc gctcttcggc aagctcttct tctccttctg 780
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<210> 6
<211> 320
<212> PRT
<213> Zea mays
    
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Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Cys Arg Gly Trp Lys
      20                      25                      30

Ser Val Tyr Cys Thr Pro Thr Arg Pro Ala Phe Lys Gly Ser Ala Pro
      35                      40                      45

Ile Asn Leu Ser Asp Arg Leu His Gln Val Leu Arg Trp Ala Leu Gly
 50                      55                      60

Ser Val Glu Ile Phe Met Ser Arg His Cys Pro Leu Trp Tyr Ala Tyr
 65                      70                      75                      80

Gly Gly Arg Leu Lys Trp Leu Glu Arg Phe Ala Tyr Thr Asn Thr Ile
      85                      90                      95

Val Tyr Pro Phe Thr Ser Ile Pro Leu Leu Ala Tyr Cys Thr Ile Pro
      100                     105                     110

Ala Val Cys Leu Leu Thr Gly Lys Phe Ile Ile Pro Thr Leu Asn Asn
      115                     120                     125

Leu Ala Ser Ile Trp Phe Ile Ala Leu Phe Leu Ser Ile Ile Ala Thr
      130                     135                     140

Ser Val Leu Glu Leu Arg Trp Ser Gly Val Ser Ile Glu Asp Trp Trp
      145                     150                     155                     160

Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Val Ser Ala His Leu Phe
      165                     170                     175

Ala Val Phe Gln Gly Phe Leu Lys Val Leu Gly Gly Val Asp Thr Ser
      180                     185                     190

Phe Thr Val Thr Ser Lys Ala Ala Gly Asp Glu Ala Asp Ala Phe Gly
      195                     200                     205

Asp Leu Tyr Leu Phe Lys Trp Thr Thr Leu Leu Val Pro Pro Thr Thr
      210                     215                     220

Leu Ile Ile Ile Asn Met Val Gly Ile Val Ala Gly Val Ser Asp Ala
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Val Asn Asn Gly Tyr Gly Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe
 245 250 255

Phe Ser Phe Trp Val Ile Val His Leu Tyr Pro Phe Leu Lys Gly Leu
 260 265 270

Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Val Leu Trp Ser Ile
 275 280 285

Leu Leu Ala Ser Ile Phe Ser Leu Val Trp Val Arg Ile Asp Pro Phe
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Ile Pro Lys Ala Lys Gly Pro Ile Leu Lys Pro Cys Gly Val Glu Cys
 305 310 315 320

<210> 7
 <211> 3786
 <212> DNA
 <213> Zea mays

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gtaaaa 3786

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<210> 8
<211> 1165
<212> PRT
<213> Zea mays

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Ala Pro Arg Gln Ala Gln Pro Pro Pro Ala Thr Ala Ala Cys Ala Cys
          20          25          30
Glu Arg Ser Pro Arg Pro Gly Asp Gln Arg Arg Gly Gly Leu Arg Ala
          35          40          45
Phe Arg Cys Ala Ala Ala Ala Gly Phe Val Arg Glu Arg Asp Pro Ala
          50          55          60
Gly Arg Gly Gly Gly Pro Glu Met Glu Ala Ser Ala Gly Leu Val Ala
          65          70          75          80
Gly Ser His Asn Arg Asn Glu Leu Val Val Ile Arg Arg Asp Arg Glu
          85          90          95
Ser Gly Ala Ala Gly Gly Gly Ala Ala Arg Arg Ala Glu Ala Pro Cys
          100          105          110
Gln Ile Cys Gly Asp Glu Val Gly Val Gly Phe Asp Gly Glu Pro Phe
          115          120          125
Val Ala Cys Asn Glu Cys Ala Phe Pro Val Cys Arg Ala Cys Tyr Glu
          130          135          140

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Tyr Glu Arg Arg Glu Gly Ser Gln Ala Cys Pro Gln Cys Arg Thr Arg
 145 150 155 160
 Tyr Lys Arg Leu Lys Gly Cys Pro Arg Val Ala Gly Asp Glu Glu Glu
 165 170 175
 Asp Gly Val Asp Asp Leu Glu Gly Glu Phe Gly Leu Gln Asp Gly Ala
 180 185 190
 Ala His Glu Asp Asp Pro Gln Tyr Val Ala Glu Ser Met Leu Arg Ala
 195 200 205
 Gln Met Ser Tyr Gly Arg Gly Gly Asp Ala His Pro Gly Phe Ser Pro
 210 215 220
 Val Pro Asn Val Pro Leu Leu Thr Asn Gly Gln Met Val Asp Asp Ile
 225 230 235 240
 Pro Pro Glu Gln His Ala Leu Val Pro Ser Tyr Met Ser Gly Gly Gly
 245 250 255
 Gly Gly Gly Lys Arg Ile His Pro Leu Pro Phe Ala Asp Pro Asn Leu
 260 265 270
 Pro Val Gln Pro Arg Ser Met Asp Pro Ser Lys Asp Leu Ala Ala Tyr
 275 280 285
 Gly Tyr Gly Ser Val Ala Trp Lys Glu Arg Met Glu Gly Trp Lys Gln
 290 295 300
 Lys Gln Glu Arg Leu Gln His Val Arg Ser Glu Gly Gly Gly Asp Trp
 305 310 315 320
 Asp Gly Asp Asp Ala Asp Leu Pro Leu Met Asp Glu Ala Arg Gln Pro
 325 330 335
 Leu Ser Arg Lys Val Pro Ile Ser Ser Ser Arg Ile Asn Pro Tyr Arg
 340 345 350
 Met Ile Ile Val Ile Arg Leu Val Val Leu Gly Phe Phe Phe His Tyr
 355 360 365
 Arg Val Met His Pro Ala Lys Asp Ala Phe Ala Leu Trp Leu Ile Ser
 370 375 380
 Val Ile Cys Glu Ile Trp Phe Ala Met Ser Trp Ile Leu Asp Gln Phe
 385 390 395 400
 Pro Lys Trp Leu Pro Ile Glu Arg Glu Thr Tyr Leu Asp Arg Leu Ser
 405 410 415
 Leu Arg Phe Asp Lys Glu Gly Gln Pro Ser Gln Leu Ala Pro Ile Asp
 420 425 430
 Phe Phe Val Ser Thr Val Asp Pro Thr Lys Glu Pro Pro Leu Val Thr
 435 440 445
 Ala Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro Val Glu Lys
 450 455 460

Val Ser Cys Tyr Val Ser Asp Asp Gly Ala Ala Met Leu Thr Phe Glu
 465 470 475 480

Ala Leu Ser Glu Thr Ser Glu Phe Ala Lys Lys Trp Val Pro Phe Ser
 485 490 495

Lys Lys Phe Asn Ile Glu Pro Arg Ala Pro Glu Trp Tyr Phe Gln Gln
 500 505 510

Lys Ile Asp Tyr Leu Lys Asp Lys Val Ala Ala Ser Phe Val Arg Glu
 515 520 525

Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn
 530 535 540

Ala Leu Val Ala Lys Ala Gln Lys Val Pro Glu Glu Gly Trp Thr Met
 545 550 555 560

Gln Asp Gly Ser Pro Trp Pro Gly Asn Asn Val Arg Asp His Pro Gly
 565 570 575

Met Ile Gln Val Phe Leu Gly Gln Ser Gly Gly Arg Asp Val Glu Gly
 580 585 590

Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly
 595 600 605

Tyr Asn His His Lys Lys Ala Gly Ala Met Asn Ala Leu Val Arg Val
 610 615 620

Ser Ala Val Leu Ser Asn Ala Ala Tyr Leu Leu Asn Leu Asp Cys Asp
 625 630 635 640

His Tyr Ile Asn Asn Ser Lys Ala Ile Lys Glu Ala Met Cys Phe Met
 645 650 655

Met Asp Pro Leu Val Gly Lys Lys Val Cys Tyr Val Gln Phe Pro Gln
 660 665 670

Arg Phe Asp Gly Ile Asp Lys Asn Asp Arg Tyr Ala Asn Arg Asn Val
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 Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Leu Lys Gly Cys Gln Arg
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Glu Lys Arg Arg Gly His Ala His His Arg Lys Ala Gly Ala Met Asn
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Ala Pro Phe Ile Leu Asp Leu Asp Cys Asp Tyr Tyr Val Asn Asn Ser
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Gln Ala Leu Arg Ala Gly Ile Cys Phe Met Ile Glu Arg Gly Gly Gly
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Val Leu Phe Asp Cys Thr Glu Leu Gly Leu Asp Gly Leu Gln Gly Pro
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 35 40 45
 Asp Gly Asp Leu Phe Val Ala Cys Asn Glu Cys Gly Phe Pro Val Cys
 50 55 60

Arg Pro Cys Tyr Glu Tyr Glu Arg Arg Glu Gly Ser His Leu Cys Pro
 65 70 75 80

Gln Cys Lys Thr Arg Tyr Lys Arg Leu Lys Gly Ser Pro Arg Val Glu
 85 90 95

Gly Asp Asp Asp Glu Glu Asp Val Asp Asp Ile Glu His Glu Phe Asn
 100 105 110

Ile Asp Glu Gln Lys Asn Lys His Gly Gln Val Ala Glu Ala Met Leu
 115 120 125

His Gly Arg Met Ser Tyr Gly Arg Gly Pro Glu Asp Asp Asp Asn Ser
 130 135 140

Gln Phe Pro Thr Pro Val Ile Ala Gly Gly Arg Ser Arg Pro Val Ser
 145 150 155 160

Gly Glu Phe Pro Ile Ser Ser Asn Ala Tyr Gly Asp Gln Met Leu Ser
 165 170 175

Ser Ser Leu His Lys Arg Val His Pro Tyr Pro Val Ser Glu Pro Gly
 180 185 190

Ser Ala Arg Trp Asp Glu Lys Lys Xaa Asp Gly Trp Lys Asp Arg Met
 195 200 205

Asp Asp Trp Lys Leu Gln Gln Gly Asn Leu Gly Pro Glu Pro Asp Glu
 210 215 220

Asp Pro Asp Ala Ala Met Leu Asp Glu Ala Arg Gln Pro Leu Ser Arg
 225 230 235 240

Lys Val Pro Ile Ala Ser Ser Lys Ile Asn Pro Tyr Arg Met Val Ile
 245 250 255

Val Ala Arg Leu Val Ile Leu Ala Phe Phe Leu Arg Tyr Arg Leu Met
 260 265 270

Asn Pro Val His Asp Ala Leu Gly Leu Trp Leu Thr Ser Ile Ile Cys
 275 280 285

Glu Ile Trp Phe Ala Phe Ser Trp Ile Leu Asp Gln Phe Pro Lys Trp
 290 295 300

Phe Pro Ile Asp Arg Glu Thr Tyr Leu Asp Arg Leu Ser Ile Arg Tyr
 305 310 315 320

Glu Arg Glu Gly Glu Pro Asn Met Leu Ala Pro Val Asp Val Phe Val
 325 330 335

Ser Thr Val Asp Pro Met Lys Glu Pro Pro Leu Val Thr Ala Asn Thr
 340 345 350

Val Leu Ser Ile Leu Ala Met Asp Tyr Pro Val Asp Lys Ile Ser Cys
 355 360 365

Tyr Ile Ser Asp Asp Gly Ala Ser Met Cys Thr Phe Glu Ser Leu Ser
 370 375 380

Glu Thr Ala Glu Phe Ala Arg Lys Trp Val Pro Phe Cys Lys Lys Phe
 385 390 395 400
 Ser Ile Glu Pro Arg Ala Pro Glu Met Tyr Phe Ser Glu Lys Ile Asp
 405 410 415
 Tyr Leu Lys Asp Lys Val Gln Pro Thr Phe Val Lys Glu Arg Arg Ala
 420 425 430
 Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn Ala Leu Val
 435 440 445
 Ala Lys Ala Gln Lys Val Pro Gln Gly Gly Trp Ile Met Gln Asp Gly
 450 455 460
 Thr Pro Trp Pro Gly Asn Asn Thr Lys Asp His Pro Gly Met Ile Gln
 465 470 475 480
 Val Phe Leu Gly Ser Ser Gly Gly Leu Asp Thr Glu Gly Asn Gln Leu
 485 490 495
 Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly Phe Gln His
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 His Lys Lys Ala Gly Ala Met Asn Ala Leu Val Arg Val Ser Ala Val
 515 520 525
 Leu Thr Asn Ala Pro Phe Met Leu Asn Leu Asp Cys Asp His Tyr Val
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 Asn Asn Ser Lys Ala Ala Arg Glu Ala Met Cys Phe Leu Met Asp Pro
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 Gln Thr Gly Lys Lys Val Cys Tyr Val Gln Phe Pro Gln Arg Phe Asp
 565 570 575
 Gly Ile Asp Thr His Asp Arg Tyr Ala Asn Arg Asn Thr Val Phe Phe
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 Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly Pro Val Tyr Val
 595 600 605
 Gly Thr Gly Cys Val Phe Arg Arg Gln Ala Leu Tyr Gly Tyr Asn Pro
 610 615 620
 Pro Lys Gly Pro Lys Arg Pro Lys Met Val Ser Cys Asp Cys Cys Pro
 625 630 635 640
 Cys Phe Gly Ser Arg Lys Lys Tyr Lys Glu Lys Asn Asp Ala Asn Gly
 645 650 655
 Glu Ala Ala Ser Leu Lys Gly Met Asp Asp Asp Lys Glu Val Leu Met
 660 665 670
 Ser Gln Met Asn Phe Glu Lys Lys Phe Gly Gln Ser Ser Ile Phe Val
 675 680 685
 Thr Ser Thr Leu Met Glu Glu Gly Gly Val Pro Pro Ser Ser Ser Pro
 690 695 700

Ala Ala Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu
 705 710 715 720
 Asp Lys Thr Glu Trp Gly Leu Glu Leu Gly Trp Ile Tyr Gly Ser Ile
 725 730 735
 Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Cys Arg Gly Trp Arg
 740 745 750
 Ser Ile Tyr Cys Met Pro Lys Arg Ala Ala Phe Lys Gly Thr Ala Pro
 755 760 765
 Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu Arg Trp Ala Leu Gly
 770 775 780
 Ser Ile Glu Ile Phe Phe Ser His His Cys Pro Leu Trp Tyr Gly Phe
 785 790 795 800
 Lys Glu Lys Lys Leu Lys Trp Leu Glu Arg Phe Ala Tyr Ala Asn Thr
 805 810 815
 Thr Val Tyr Pro Phe Thr Ser Ile Pro Leu Val Ala Tyr Cys Ile Leu
 820 825 830
 Pro Ala Val Cys Leu Leu Thr Asp Lys Phe Ile Met Pro Pro Ile Ser
 835 840 845
 Thr Phe Ala Gly Leu Tyr Phe Val Ala Leu Phe Ser Ser Ile Ile Ala
 850 855 860
 Thr Gly Ile Leu Glu Leu Lys Trp Ser Gly Val Ser Ile Glu Glu Trp
 865 870 875 880
 Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Val Ser Ala His Leu
 885 890 895
 Phe Ala Val Ile Gln Gly Leu Leu Lys Val Leu Ala Gly Ile Asp Thr
 900 905 910
 Asn Phe Thr Val Thr Ser Lys Ala Thr Asp Asp Glu Glu Phe Gly Glu
 915 920 925
 Leu Tyr Thr Phe Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Ile
 930 935 940
 Leu Ile Ile Asn Ile Val Gly Val Val Ala Gly Ile Ser Asp Ala Ile
 945 950 955 960
 Asn Asn Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe
 965 970 975
 Ser Phe Trp Val Ile Val His Leu Tyr Pro Phe Leu Lys Gly Leu Met
 980 985 990
 Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Val Ile Trp Ser Val Leu
 995 1000 1005
 Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Arg Ile Asp Pro Phe Val
 1010 1015 1020

Leu Lys Thr Lys Gly Pro Asp Thr Lys Leu Cys Gly Ile Asn Cys
 1025 1030 1035

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 <212> DNA
 <213> Glycine max

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 ctggatacag atggaaatga gctgcctaga cttgtttatg tttctcgtga gaagcgacca 180
 ggcttccaac atcacaagaa ggctggagct atgaatgctt tgattcgagt ttctgctgtc 240
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 gaagatgttg aaacctaacat tattgtaaag agttgttgcg gttctagaaa gaagggaaa 600
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 cccatattta atatggaaga catagaggag ggtgttgaag gtatgatga tgaaggaga 720
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 <211> 610
 <212> PRT
 <213> Glycine max

<400> 16
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Val Phe Leu Gly His Ser Gly Gly Leu Asp Thr Asp Gly Asn Glu Leu
 35 40 45

Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly Phe Gln His
 50 55 60

His Lys Lys Ala Gly Ala Met Asn Ala Leu Ile Arg Val Ser Ala Val
 65 70 75 80

Leu Thr Asn Gly Ala Tyr Leu Leu Asn Val Asp Cys Asp His Tyr Phe
 85 90 95

Asn Asn Ser Lys Ala Leu Lys Glu Ala Met Cys Phe Met Met Asp Pro
 100 105 110

Val Leu Gly Lys Lys Thr Cys Tyr Val Gln Phe Pro Gln Arg Phe Asp
 115 120 125

Gly Ile Asp Leu His Asp Arg Tyr Ala Asn Arg Asn Ile Val Phe Phe
 130 135 140

Asp Ile Asn Met Lys Gly Gln Asp Gly Val Gln Gly Pro Val Tyr Val
 145 150 155 160

Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala Leu Tyr Gly Tyr Asp Pro
 165 170 175

Val Leu Thr Glu Glu Asp Leu Glu Pro Asn Ile Ile Val Lys Ser Cys
 180 185 190

Cys Gly Ser Arg Lys Lys Gly Lys Gly Gly Asn Lys Lys Tyr Ser Asp
 195 200 205

Lys Lys Lys Ala Met Gly Arg Thr Glu Ser Thr Val Pro Ile Phe Asn
 210 215 220

Met Glu Asp Ile Glu Glu Gly Val Glu Gly Tyr Asp Asp Glu Arg Thr
 225 230 235 240

Leu Leu Met Ser Gln Lys Ser Leu Glu Lys Arg Phe Gly Gln Ser Pro
 245 250 255

Val Phe Ile Ala Ala Thr Phe Met Glu Gln Gly Gly Ile Pro Pro Ser
 260 265 270

Thr Asn Pro Ala Thr Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys
 275 280 285

Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys Glu Ile Gly Trp Ile Tyr
 290 295 300

Gly Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Ala Arg
 305 310 315 320

Gly Trp Ile Ser Ile Tyr Cys Met Pro Pro Arg Pro Ala Phe Lys Gly
 325 330 335

Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu Arg Trp
 340 345 350

Ala Leu Gly Ser Ile Glu Ile Phe Leu Ser Arg His Cys Pro Leu Trp
 355 360 365

Tyr Gly Tyr Asn Gly Lys Leu Lys Pro Leu Met Arg Leu Ala Tyr Ile
 370 375 380

Asn Thr Ile Val Tyr Pro Phe Thr Ser Ile Pro Leu Ile Ala Tyr Cys
 385 390 395 400

Thr Leu Pro Ala Phe Cys Leu Leu Thr Asn Lys Phe Ile Ile Pro Glu
 405 410 415

Ile Ser Asn Phe Ala Ser Met Trp Phe Ile Leu Leu Phe Val Ser Ile
 420 425 430

Phe Thr Thr Ser Ile Leu Glu Leu Arg Trp Ser Gly Val Ser Ile Glu
 435 440 445

Asp Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Thr Ser Ala
 450 455 460

His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu Ala Gly Ile
 465 470 475 480

Asp Thr Asn Phe Thr Val Thr Ser Lys Ala Ser Asp Glu Asp Gly Asp
 485 490 495

Phe Ala Glu Leu Tyr Val Phe Lys Trp Thr Ser Leu Leu Ile Pro Pro
 500 505 510

Thr Thr Val Leu Ile Val Asn Leu Val Gly Ile Val Ala Gly Val Ser
 515 520 525

Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys
 530 535 540

Leu Phe Phe Ala Ile Trp Val Ile Ala His Leu Tyr Pro Phe Leu Lys
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Gly Leu Leu Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp
 565 570 575

Ser Val Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Arg Ile Asp
 580 585 590

Pro Phe Thr Ser Asp Ser Asn Lys Leu Thr Asn Gly Gln Cys Gly Ile
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Asn Cys
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 <211> 2890
 <212> DNA
 <213> Glycine max

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<212> PRT
<213> Glycine max
    
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Pro Lys Trp Tyr Pro Ile Gln Arg Glu Thr Tyr Leu Asp Arg Leu Ser
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Leu Arg Tyr Glu Lys Glu Gly Lys Pro Ser Glu Leu Ser Ser Val Asp
 50 55 60

Val Phe Val Ser Thr Val Asp Pro Met Lys Glu Pro Pro Leu Ile Thr
 65 70 75 80

Ala Asn Thr Val Leu Ser Ile Leu Ala Val Asp Tyr Pro Val Asp Lys
 85 90 95

Val Ala Cys Tyr Val Ser Asp Asp Gly Ala Ala Met Leu Thr Phe Glu
 100 105 110

Ala Leu Ser Glu Thr Ser Glu Phe Ala Arg Arg Trp Val Pro Phe Cys
 115 120 125

Lys Lys Tyr Asn Ile Glu Pro Arg Ala Pro Glu Trp Tyr Phe Gly Gln
 130 135 140

Lys Met Asp Tyr Leu Lys Asn Lys Val His Pro Ala Phe Val Arg Glu
 145 150 155 160

Arg Arg Ala Met Lys Arg Asp Tyr Glu Glu Phe Lys Val Arg Ile Asn
 165 170 175

Ser Leu Val Ala Thr Ala Gln Lys Val Pro Glu Asp Gly Trp Thr Met
 180 185 190

Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Val Arg Asp His Pro Gly
 195 200 205

Met Ile Gln Val Phe Leu Gly Gln Asp Gly Val Arg Asp Val Glu Gly
 210 215 220

Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly
 225 230 235 240

Phe Asp His His Lys Lys Ala Gly Ala Met Asn Ala Leu Val Arg Ala
 245 250 255

Ser Ala Ile Ile Thr Asn Ala Pro Tyr Leu Leu Asn Val Asp Cys Asp
 260 265 270

His Tyr Ile Asn Asn Ser Lys Ala Leu Arg Glu Ala Met Cys Phe Met
 275 280 285

Met Asp Pro Gln Leu Gly Lys Lys Val Cys Tyr Val Gln Phe Pro Gln
 290 295 300

Arg Phe Asp Gly Ile Asp Arg His Asp Arg Tyr Ser Asn Arg Asn Val
 305 310 315 320

Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly Pro
 325 330 335

Ile Tyr Val Gly Thr Gly Cys Val Phe Arg Arg Tyr Ala Leu Tyr Gly
 340 345 350

Tyr Asp Ala Pro Ala Lys Lys Lys Pro Pro Ser Lys Thr Cys Asn Cys
 355 360 365
 Trp Pro Lys Trp Cys Cys Leu Cys Cys Gly Ser Arg Lys Lys Lys Asn
 370 375 380
 Ala Asn Ser Lys Lys Glu Lys Lys Arg Lys Val Lys His Ser Glu Ala
 385 390 395 400
 Ser Lys Gln Ile His Ala Leu Glu Asn Ile Glu Ala Gly Asn Glu Gly
 405 410 415
 Thr Asn Asn Glu Lys Thr Ser Asn Leu Thr Gln Thr Lys Leu Glu Lys
 420 425 430
 Arg Phe Gly Gln Ser Pro Val Phe Val Ala Ser Thr Leu Leu Asp Asp
 435 440 445
 Gly Gly Val Pro His Gly Val Ser Pro Ala Ser Leu Leu Lys Glu Ala
 450 455 460
 Ile Gln Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys
 465 470 475 480
 Glu Val Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly
 485 490 495
 Phe Lys Met His Cys His Gly Trp Arg Ser Val Tyr Cys Ile Pro Lys
 500 505 510
 Arg Pro Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu
 515 520 525
 His Gln Val Leu Arg Trp Ala Leu Gly Ser Val Glu Ile Phe Phe Ser
 530 535 540
 Arg His Cys Pro Ile Trp Tyr Gly Tyr Gly Gly Leu Lys Leu Leu
 545 550 555 560
 Glu Arg Phe Ser Tyr Ile Asn Ser Val Val Tyr Pro Trp Thr Ser Leu
 565 570 575
 Pro Leu Leu Val Tyr Cys Thr Leu Pro Ala Ile Cys Leu Leu Thr Gly
 580 585 590
 Lys Phe Ile Val Pro Glu Ile Ser Asn Tyr Ala Ser Leu Val Phe Met
 595 600 605
 Ala Leu Phe Ile Ser Ile Ala Ala Thr Gly Ile Leu Glu Met Gln Trp
 610 615 620
 Gly Gly Val Ser Ile Asp Asp Trp Trp Arg Asn Glu Gln Phe Trp Val
 625 630 635 640
 Ile Gly Gly Val Ser Ser His Leu Phe Ala Leu Phe Gln Gly Leu Leu
 645 650 655
 Lys Val Leu Ala Gly Val Asn Thr Asn Phe Thr Val Thr Ser Lys Ala
 660 665 670

Ala Asp Asp Gly Glu Phe Ser Glu Leu Tyr Ile Phe Lys Trp Thr Ser
 675 680 685
 Leu Leu Ile Pro Pro Met Thr Leu Leu Ile Met Asn Ile Val Gly Val
 690 700
 Val Val Gly Ile Ser Asp Ala Ile Asn Asn Gly Tyr Asp Ser Trp Gly
 705 710 715 720
 Pro Leu Phe Gly Arg Leu Phe Phe Ala Leu Trp Val Ile Leu His Leu
 725 730 735
 Tyr Pro Phe Leu Lys Gly Leu Leu Gly Lys Gln Asp Arg Met Pro Thr
 740 745 750
 Ile Ile Leu Val Trp Ser Ile Leu Leu Ala Ser Ile Leu Thr Leu Met
 755 760 765
 Trp Val Arg Ile Asn Pro Phe Val Ser Arg Asp Gly Pro Val Leu Glu
 770 775 780
 Ile Cys Gly Leu Asn Cys Asp Glu Ser
 785 790

<210> 19
 <211> 1742
 <212> DNA
 <213> Triticum aestivum

<220>
 <221> unsure
 <222> (9)

<220>
 <221> unsure
 <222> (271)

<400> 19
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 actgtctttt ttgatattaa cttgaggggc cttgacggca ttcaaggacc agtttatgtg 180
 ggaactggtt gtgttttcaa cagaacggct atctatgggt atgagccccc aattaaggcg 240
 aagaagccag gtttcttggc atcattatgt nggggcaaga agaaggcaag caagtcaaag 300
 aaaaggagct cagataagaa aaagtccaac aagcatgtgg acagtctgt tccagtattc 360
 aatctcgaag acatagagga ggggtgtgaa ggtgctgggt ttgatgatga gaaatcagtt 420
 ctcatgtctc aaatgagctt agagaagaga tttggccagt cagcagcatt tgttgcctcc 480
 actctgatgg aatatgggtg tgttcctcag tcgtccactc cagaatctct ttgaaagaa 540
 gctatccatg tcataagttg tggctatgag gacaagtctg aatggggaac tgagattggt 600
 tggatctatg gatctgtcac agaagatatt ctaactggat tcaagatgca cgcaagaggc 660
 tggcgttcaa tctattgcat gccaagcgc ccagctttca agggatctgc cccatcaat 720
 ctttcagatc gtctgaatca agtgctgctg tgggctcttg gttctgttga aattcttttc 780
 agccggcatt gccccttatg gtatggctac ggagggcgcc tcaagttcct ggagagattc 840
 gcttacatca acaccacat ttaccacta acctctctcc cgcttctagt ctattgtata 900
 ttgcctgcta tctgtctgct cactggaaag ttcattatgc cagagattag caacttggcc 960
 agtatctggt tcattgcgct cttcctttca attttcgcca ctggatcct tgagatgagg 1020
 tggagtgtg ttggcattga cgagtgggtg agaatgaac agttctgggt cattggaggt 1080
 atctctgcc atctgttgc cgtctttcag ggtcttctga agtgcttgc aggtatcgac 1140
 accaacttca ctgtcacctc aaaggctaag gatgaagaag gcgactttgc tgagctctac 1200
 atgttcaagt ggacgacgct tcttatccct ccgacgacca ttttgatcat taacatggtc 1260
 ggtgtcgttg ctggtacctc ctacgccatc aacagtgggt accaatcatg ggggccgctc 1320

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tttgggaagc tcttctttgc cttctgggtg attgttcaact tataccatt cctcaagggt 1380
cttatgggca ggcaaaaccg cacaccgacg attgtcatcg tctgggctgt cctcctcgct 1440
tctatcttct ccttgctgtg ggttcgtgtt gatccattca ctaccctct cgctggccca 1500
aatatccaaa cctgtggcat caactgctag gaaagtggga gttttagag acagaaaata 1560
taacagtgat cgagcgacca cctgtggagc cagagaatat ttatgttggg gttgtgaatt 1620
actacgtttg agaaaattgt caaaattgag aaaacacatt tgtaaataga tgtaatagac 1680
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aa

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<210> 20
<211> 506
<212> PRT
<213> Triticum aestivum

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<220>
<221> UNSURE
<222> (88)

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Tyr Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Arg Asn Asp Arg
          20          25          30
Tyr Ala Asn Arg Asn Thr Val Phe Phe Asp Ile Asn Leu Arg Gly Leu
          35          40          45
Asp Gly Ile Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Val Phe Asn
          50          55          60
Arg Thr Ala Ile Tyr Gly Tyr Glu Pro Pro Ile Lys Ala Lys Lys Pro
          65          70          75          80
Gly Phe Leu Ala Ser Leu Cys Xaa Gly Lys Lys Lys Ala Ser Lys Ser
          85          90          95
Lys Lys Arg Ser Ser Asp Lys Lys Lys Ser Asn Lys His Val Asp Ser
          100          105          110
Ser Val Pro Val Phe Asn Leu Glu Asp Ile Glu Glu Gly Val Glu Gly
          115          120          125
Ala Gly Phe Asp Asp Glu Lys Ser Val Leu Met Ser Gln Met Ser Leu
          130          135          140
Glu Lys Arg Phe Gly Gln Ser Ala Ala Phe Val Ala Ser Thr Leu Met
          145          150          155          160
Glu Tyr Gly Gly Val Pro Gln Ser Ser Thr Pro Glu Ser Leu Leu Lys
          165          170          175
Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu Asp Lys Ser Glu Trp
          180          185          190
Gly Thr Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu
          195          200          205
Thr Gly Phe Lys Met His Ala Arg Gly Trp Arg Ser Ile Tyr Cys Met
          210          215          220

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Pro Lys Arg Pro Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp
 225 230 235 240

Arg Leu Asn Gln Val Leu Arg Trp Ala Leu Gly Ser Val Glu Ile Leu
 245 250 255

Phe Ser Arg His Cys Pro Leu Trp Tyr Gly Tyr Gly Gly Arg Leu Lys
 260 265 270

Phe Leu Glu Arg Phe Ala Tyr Ile Asn Thr Thr Ile Tyr Pro Leu Thr
 275 280 285

Ser Leu Pro Leu Leu Val Tyr Cys Ile Leu Pro Ala Ile Cys Leu Leu
 290 295 300

Thr Gly Lys Phe Ile Met Pro Glu Ile Ser Asn Leu Ala Ser Ile Trp
 305 310 315 320

Phe Ile Ala Leu Phe Leu Ser Ile Phe Ala Thr Gly Ile Leu Glu Met
 325 330 335

Arg Trp Ser Gly Val Gly Ile Asp Glu Trp Trp Arg Asn Glu Gln Phe
 340 345 350

Trp Val Ile Gly Gly Ile Ser Ala His Leu Phe Ala Val Phe Gln Gly
 355 360 365

Leu Leu Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser
 370 375 380

Lys Ala Asn Asp Glu Glu Gly Asp Phe Ala Glu Leu Tyr Met Phe Lys
 385 390 395 400

Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Ile Leu Ile Ile Asn Met
 405 410 415

Val Gly Val Val Ala Gly Thr Ser Tyr Ala Ile Asn Ser Gly Tyr Gln
 420 425 430

Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Phe Trp Val Ile
 435 440 445

Val His Leu Tyr Pro Phe Leu Lys Gly Leu Met Gly Arg Gln Asn Arg
 450 455 460

Thr Pro Thr Ile Val Ile Val Trp Ala Val Leu Leu Ala Ser Ile Phe
 465 470 475 480

Ser Leu Leu Trp Val Arg Val Asp Pro Phe Thr Thr Arg Leu Ala Gly
 485 490 495

Pro Asn Ile Gln Thr Cys Gly Ile Asn Cys
 500 505

<210> 21
 <211> 1029
 <212> DNA
 <213> Triticum aestivum

<400> 21
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 gctcgtgccg tcctacatga gcggcggcgg cggcgggggc aagaggatcc acccgctccc 120
 tttcgagat cccaaccttc cagtgaacc gagatccatg gaccctcca aggatctggc 180
 cgcctacgga tatggcagcg tggcctgga ggagagaatg gagggtgga agcagaagca 240
 ggagcgctg cagcatgtca ggagcgagg tggcggatg tgggatggcg acgatgcaga 300
 tctgccacta atggatgaag ctaggcagcc attgtccaga aaagtcccta tatcatcaag 360
 ccgaattaat ccctacagga tgattatcgt tacccggtg gtggttttgg gtttcttctt 420
 cactaccga gtgatgcac cggcgaaaga tgcatttgca ttgtggctca tatctgtaat 480
 ctgtgaaatc tggtttgca tgcctgtat tcttgatcag ttcccaaagt ggtttccaat 540
 cgagagagag acttacctgg accgtttgtc actaaggttt gacaaggaag gtcaaccctc 600
 tcagcttgct ccaatcgact tctttgtcag tacggttgat cccacaaagg aacctccctt 660
 ggtcacagcg aacctgtcc ttccatcct ttctgtgat tatccggttg agaaggtctc 720
 ctgctatggt tctgatgatg gtgctgaat gcttacgttt gaagcattgt ctgaaacatc 780
 tgaatttgca aagaaatggg ttcctttcag caaaaagttt aatatcgagc ctctgtctcc 840
 tgagtggtag ttccaacaga agatagacta cctgaaagac aaggttgctg cttcatttgt 900
 tagggagagg agggcgatga agagagaata cgaggaattc aaggtaagga tcaatgcctt 960
 ggttgcaaaa gcccaaaagg ttcctgagga aggatggaca atgcaagatg gaagcccctg 1020
 gcctggaaa 1029

<210> 22
 <211> 340
 <212> PRT
 <213> Triticum aestivum

<400> 22
 Pro Leu Leu Thr Asn Gly Gln Met Val Asp Asp Ile Pro Pro Glu Gln
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 His Ala Leu Val Pro Ser Tyr Met Ser Gly Gly Gly Gly Gly Lys
 20 25 30
 Arg Ile His Pro Leu Pro Phe Ala Asp Pro Asn Leu Pro Val Gln Pro
 35 40 45
 Arg Ser Met Asp Pro Ser Lys Asp Leu Ala Ala Tyr Gly Tyr Gly Ser
 50 55 60
 Val Ala Trp Lys Glu Arg Met Glu Gly Trp Lys Gln Lys Gln Glu Arg
 65 70 75 80
 Leu Gln His Val Arg Ser Glu Gly Gly Gly Asp Trp Asp Gly Asp Asp
 85 90 95
 Ala Asp Leu Pro Leu Met Asp Glu Ala Arg Gln Pro Leu Ser Arg Lys
 100 105 110
 Val Pro Ile Ser Ser Ser Arg Ile Asn Pro Tyr Arg Met Ile Ile Val
 115 120 125
 Ile Arg Leu Val Val Leu Gly Phe Phe Phe His Tyr Arg Val Met His
 130 135 140
 Pro Ala Lys Asp Ala Phe Ala Leu Trp Leu Ile Ser Val Ile Cys Glu
 145 150 155 160
 Ile Trp Phe Ala Met Ser Cys Ile Leu Asp Gln Phe Pro Lys Trp Phe
 165 170 175

Pro Ile Glu Arg Glu Thr Tyr Leu Asp Arg Leu Ser Leu Arg Phe Asp
 180 185 190

Lys Glu Gly Gln Pro Ser Gln Leu Ala Pro Ile Asp Phe Phe Val Ser
 195 200 205

Thr Val Asp Pro Thr Lys Glu Pro Pro Leu Val Thr Ala Asn Thr Val
 210 215 220

Leu Ser Ile Leu Ser Val Asp Tyr Pro Val Glu Lys Val Ser Cys Tyr
 225 230 235 240

Val Ser Asp Asp Gly Ala Ala Met Leu Thr Phe Glu Ala Leu Ser Glu
 245 250 255

Thr Ser Glu Phe Ala Lys Lys Trp Val Pro Phe Ser Lys Lys Phe Asn
 260 265 270

Ile Glu Pro Arg Ala Pro Glu Trp Tyr Phe Gln Gln Lys Ile Asp Tyr
 275 280 285

Leu Lys Asp Lys Val Ala Ala Ser Phe Val Arg Glu Arg Arg Ala Met
 290 295 300

Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn Ala Leu Val Ala
 305 310 315 320

Lys Ala Gln Lys Val Pro Glu Glu Gly Trp Thr Met Gln Asp Gly Ser
 325 330 335

Pro Trp Pro Gly
 340

<210> 23
 <211> 1081
 <212> PRT
 <213> Arabidopsis thaliana

<400> 23
 Met Glu Ala Ser Ala Gly Leu Val Ala Gly Ser Tyr Arg Arg Asn Glu
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Leu Val Arg Ile Arg His Glu Ser Asp Gly Gly Thr Lys Pro Leu Lys
 20 25 30

Asn Met Asn Gly Gln Ile Cys Gln Ile Cys Gly Asp Asp Val Gly Leu
 35 40 45

Ala Glu Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala Phe Pro
 50 55 60

Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Asp Gly Thr Gln Cys
 65 70 75 80

Cys Pro Gln Cys Lys Thr Arg Phe Arg Arg His Arg Gly Ser Pro Arg
 85 90 95

Val Glu Gly Asp Glu Asp Glu Asp Asp Val Asp Asp Ile Glu Asn Glu
 100 105 110

Phe Asn Tyr Ala Gln Gly Ala Asn Lys Ala Arg His Gln Arg His Gly
 115 120 125
 Glu Glu Phe Ser Ser Ser Ser Arg His Glu Ser Gln Pro Ile Pro Leu
 130 135 140
 Leu Thr His Gly His Thr Val Ser Gly Glu Ile Arg Thr Pro Asp Thr
 145 150 155 160
 Gln Ser Val Arg Thr Thr Ser Gly Pro Leu Gly Pro Ser Asp Arg Asn
 165 170 175
 Ala Ile Ser Ser Pro Tyr Ile Asp Pro Arg Gln Pro Val Pro Val Arg
 180 185 190
 Ile Val Asp Pro Ser Lys Asp Leu Asn Ser Tyr Gly Leu Gly Asn Val
 195 200 205
 Asp Trp Lys Glu Arg Val Glu Gly Trp Lys Leu Lys Gln Glu Lys Asn
 210 215 220
 Met Leu Gln Met Thr Gly Lys Tyr His Glu Gly Lys Gly Gly Glu Ile
 225 230 235 240
 Glu Gly Thr Gly Ser Asn Gly Glu Glu Leu Gln Met Ala Asp Asp Thr
 245 250 255
 Arg Leu Pro Met Ser Arg Val Val Pro Ile Pro Ser Ser Arg Leu Thr
 260 265 270
 Pro Tyr Arg Val Val Ile Ile Leu Arg Leu Ile Ile Leu Cys Phe Phe
 275 280 285
 Leu Gln Tyr Arg Thr Thr His Pro Val Lys Asn Ala Tyr Pro Leu Trp
 290 295 300
 Leu Thr Ser Val Ile Cys Glu Ile Trp Phe Ala Phe Ser Trp Leu Leu
 305 310 315 320
 Asp Gln Phe Pro Lys Trp Tyr Pro Ile Asn Arg Glu Thr Tyr Leu Asp
 325 330 335
 Arg Leu Ala Ile Arg Tyr Asp Arg Asp Gly Glu Pro Ser Gln Leu Val
 340 345 350
 Pro Val Asp Val Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro
 355 360 365
 Leu Val Thr Ala Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro
 370 375 380
 Val Asp Lys Val Ala Cys Tyr Val Ser Asp Asp Gly Ser Ala Met Leu
 385 390 395 400
 Thr Phe Glu Ser Leu Ser Glu Thr Ala Glu Phe Ala Lys Lys Trp Val
 405 410 415
 Pro Phe Cys Lys Lys Phe Asn Ile Glu Pro Arg Ala Pro Glu Phe Tyr
 420 425 430

Phe Ala Gln Lys Ile Asp Tyr Leu Lys Asp Lys Ile Gln Pro Ser Phe
 435 440 445
 Val Lys Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val
 450 455 460
 Arg Ile Asn Ala Leu Val Ala Lys Ala Gln Lys Ile Pro Glu Glu Gly
 465 470 475 480
 Trp Thr Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Thr Arg Asp
 485 490 495
 His Pro Gly Met Ile Gln Val Phe Leu Gly His Ser Gly Gly Leu Asp
 500 505 510
 Thr Asp Gly Asn Glu Leu Pro Arg Leu Ile Tyr Val Ser Arg Glu Lys
 515 520 525
 Arg Pro Gly Phe Gln His His Lys Lys Ala Gly Ala Met Asn Ala Leu
 530 535 540
 Ile Arg Val Ser Ala Val Leu Thr Asn Gly Ala Tyr Leu Leu Asn Val
 545 550 555 560
 Asp Cys Asp His Tyr Phe Asn Asn Ser Lys Ala Ile Lys Glu Ala Met
 565 570 575
 Cys Phe Met Met Asp Pro Ala Ile Gly Lys Lys Cys Cys Tyr Val Gln
 580 585 590
 Phe Pro Gln Arg Phe Asp Gly Ile Asp Leu His Asp Arg Tyr Ala Asn
 595 600 605
 Arg Asn Ile Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile
 610 615 620
 Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala
 625 630 635 640
 Leu Tyr Gly Tyr Asp Pro Val Leu Thr Glu Glu Asp Leu Glu Pro Asn
 645 650 655
 Ile Ile Val Lys Ser Cys Cys Gly Ser Arg Lys Lys Gly Lys Ser Ser
 660 665 670
 Lys Lys Tyr Asn Tyr Glu Lys Arg Arg Gly Ile Asn Arg Ser Asp Ser
 675 680 685
 Asn Ala Pro Leu Phe Asn Met Glu Asp Ile Asp Glu Gly Phe Glu Gly
 690 695 700
 Tyr Asp Asp Glu Arg Ser Ile Leu Met Ser Gln Arg Ser Val Glu Lys
 705 710 715 720
 Arg Phe Gly Gln Ser Pro Val Phe Ile Ala Ala Thr Phe Met Glu Gln
 725 730 735
 Gly Gly Ile Pro Pro Thr Thr Asn Pro Ala Thr Leu Leu Lys Glu Ala
 740 745 750

Ile His Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys
 755 760 765

Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly
 770 775 780

Phe Lys Met His Ala Arg Gly Trp Ile Ser Ile Tyr Cys Asn Pro Pro
 785 790 795 800

Arg Pro Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu
 805 810 815

Asn Gln Val Leu Arg Trp Ala Leu Gly Ser Ile Glu Ile Leu Leu Ser
 820 825 830

Arg His Cys Pro Ile Trp Tyr Gly Tyr His Gly Arg Leu Arg Leu Leu
 835 840 845

Glu Arg Ile Ala Tyr Ile Asn Thr Ile Val Tyr Pro Ile Thr Ser Ile
 850 855 860

Pro Leu Ile Ala Tyr Cys Ile Leu Pro Ala Phe Cys Leu Ile Thr Asp
 865 870 875 880

Arg Phe Ile Ile Pro Glu Ile Ser Asn Tyr Ala Ser Ile Trp Phe Ile
 885 890 895

Leu Leu Phe Ile Ser Ile Ala Val Thr Gly Ile Leu Glu Leu Arg Trp
 900 905 910

Ser Gly Val Ser Ile Glu Asp Trp Trp Arg Asn Glu Gln Phe Trp Val
 915 920 925

Ile Gly Gly Thr Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu
 930 935 940

Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser Lys Ala
 945 950 955 960

Thr Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr Ile Phe Lys Trp Thr
 965 970 975

Ala Leu Leu Ile Pro Pro Thr Thr Val Leu Leu Val Asn Leu Ile Gly
 980 985 990

Ile Val Ala Gly Val Ser Tyr Ala Val Asn Ser Gly Tyr Gln Ser Trp
 995 1000 1005

Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Leu Trp Val Ile Ala His
 1010 1015 1020

Leu Tyr Pro Phe Leu Lys Gly Leu Leu Gly Arg Gln Asn Arg Thr Pro
 1025 1030 1035 1040

Thr Ile Val Ile Val Trp Ser Val Leu Leu Ala Ser Ile Phe Ser Leu
 1045 1050 1055

Leu Trp Val Arg Ile Asn Pro Phe Val Asp Ala Asn Pro Asn Ala Asn
 1060 1065 1070

Asn Phe Asn Gly Lys Gly Gly Val Phe
 1075 1080

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 <212> PRT
 <213> Arabidopsis thaliana

<400> 24
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 Glu Leu Ser Gly Gln Thr Cys Gln Ile Cys Gly Asp Glu Ile Glu Leu
 35 40 45
 Thr Val Ser Ser Glu Leu Phe Val Ala Cys Asn Glu Cys Ala Phe Pro
 50 55 60
 Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Arg Glu Gly Asn Gln Ala
 65 70 75 80
 Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Ile Lys Gly Ser Pro Arg
 85 90 95
 Val Asp Gly Asp Asp Glu Glu Glu Glu Asp Ile Asp Asp Leu Glu Tyr
 100 105 110
 Glu Phe Asp His Gly Met Asp Pro Glu His Ala Ala Glu Ala Ala Leu
 115 120 125
 Ser Ser Arg Leu Asn Thr Gly Arg Gly Gly Leu Asp Ser Ala Pro Pro
 130 135 140
 Gly Ser Gln Ile Pro Leu Leu Thr Tyr Cys Asp Glu Asp Ala Asp Met
 145 150 155 160
 Tyr Ser Asp Arg His Ala Leu Ile Val Pro Pro Ser Thr Gly Tyr Gly
 165 170 175
 Asn Arg Val Tyr Pro Ala Pro Phe Thr Asp Ser Ser Ala Pro Pro Gln
 180 185 190
 Ala Arg Ser Met Val Pro Gln Lys Asp Ile Ala Glu Tyr Gly Tyr Gly
 195 200 205
 Ser Val Ala Trp Lys Asp Arg Met Glu Val Trp Lys Arg Arg Gln Gly
 210 215 220
 Glu Lys Leu Gln Val Ile Lys His Glu Gly Gly Asn Asn Gly Arg Gly
 225 230 235 240
 Ser Asn Asp Asp Asp Glu Leu Asp Asp Pro Asp Met Pro Met Met Asp
 245 250 255
 Glu Gly Arg Gln Pro Leu Ser Arg Lys Leu Pro Ile Arg Ser Ser Arg
 260 265 270

Ile Asn Pro Tyr Arg Met Leu Ile Leu Cys Arg Leu Ala Ile Leu Gly
 275 280 285

Leu Phe Phe His Tyr Arg Ile Leu His Pro Val Asn Asp Ala Tyr Gly
 290 295 300

Leu Trp Leu Thr Ser Val Ile Cys Glu Ile Trp Phe Ala Val Ser Trp
 305 310 315 320

Ile Leu Asp Gln Phe Pro Lys Trp Tyr Pro Ile Glu Arg Glu Thr Tyr
 325 330 335

Leu Asp Arg Leu Ser Leu Arg Tyr Glu Lys Glu Gly Lys Pro Ser Gly
 340 345 350

Leu Ala Pro Val Asp Val Phe Val Ser Thr Val Asp Pro Leu Lys Glu
 355 360 365

Pro Pro Leu Ile Thr Ala Asn Thr Val Leu Ser Ile Leu Ala Val Asp
 370 375 380

Tyr Pro Val Asp Lys Val Ala Cys Tyr Val Ser Asp Asp Gly Ala Ala
 385 390 395 400

Met Leu Thr Phe Glu Ala Leu Ser Asp Thr Ala Glu Phe Ala Arg Lys
 405 410 415

Trp Val Pro Phe Cys Lys Lys Phe Asn Ile Glu Pro Arg Ala Pro Glu
 420 425 430

Trp Tyr Phe Ser Gln Lys Met Asp Tyr Leu Lys Asn Lys Val His Pro
 435 440 445

Ala Phe Val Arg Glu Arg Arg Ala Met Lys Arg Asp Tyr Glu Glu Phe
 450 455 460

Lys Val Lys Ile Asn Ala Leu Val Ala Thr Ala Gln Lys Val Pro Glu
 465 470 475 480

Glu Gly Trp Thr Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Val
 485 490 495

Arg Asp His Pro Gly Met Ile Gln Val Phe Leu Gly His Ser Gly Val
 500 505 510

Arg Asp Thr Asp Gly Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg
 515 520 525

Glu Lys Arg Pro Gly Phe Asp His His Lys Lys Ala Gly Ala Met Asn
 530 535 540

Ser Leu Ile Arg Val Ser Ala Val Leu Ser Asn Ala Pro Tyr Leu Leu
 545 550 555 560

Asn Val Asp Cys Asp His Tyr Ile Asn Asn Ser Lys Ala Ile Arg Glu
 565 570 575

Ser Met Cys Phe Met Met Asp Pro Gln Ser Gly Lys Lys Val Cys Tyr
 580 585 590

Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Arg His Asp Arg Tyr
 595 600 605
 Ser Asn Arg Asn Val Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp
 610 615 620
 Gly Ile Gln Gly Pro Ile Tyr Val Gly Thr Gly Cys Val Phe Arg Arg
 625 630 635 640
 Gln Ala Leu Tyr Gly Phe Asp Ala Pro Lys Lys Lys Lys Pro Pro Gly
 645 650 655
 Lys Thr Cys Asn Cys Trp Pro Lys Trp Cys Cys Leu Cys Cys Gly Leu
 660 665 670
 Arg Lys Lys Ser Lys Thr Lys Ala Lys Asp Lys Lys Thr Asn Thr Lys
 675 680 685
 Glu Thr Ser Lys Gln Ile His Ala Leu Glu Asn Val Asp Glu Gly Val
 690 695 700
 Ile Val Pro Val Ser Asn Val Glu Lys Arg Ser Glu Ala Thr Gln Leu
 705 710 715 720
 Lys Leu Glu Lys Lys Phe Gly Gln Ser Pro Val Phe Val Ala Ser Ala
 725 730 735
 Val Leu Gln Asn Gly Gly Val Pro Arg Asn Ala Ser Pro Ala Cys Leu
 740 745 750
 Leu Arg Glu Ala Ile Gln Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr
 755 760 765
 Glu Trp Gly Lys Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp
 770 775 780
 Ile Leu Thr Gly Phe Lys Met His Cys His Gly Trp Arg Ser Val Tyr
 785 790 795 800
 Cys Met Pro Lys Arg Ala Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu
 805 810 815
 Ser Asp Arg Leu His Gln Val Leu Arg Trp Ala Leu Gly Ser Val Glu
 820 825 830
 Ile Phe Leu Ser Arg His Cys Pro Ile Trp Tyr Gly Tyr Gly Gly Gly
 835 840 845
 Leu Lys Trp Leu Glu Arg Phe Ser Tyr Ile Asn Ser Val Val Tyr Pro
 850 855 860
 Trp Thr Ser Leu Pro Leu Ile Val Tyr Cys Ser Leu Pro Ala Val Cys
 865 870 875 880
 Leu Leu Thr Gly Lys Phe Ile Val Pro Glu Ile Ser Asn Tyr Ala Gly
 885 890 895
 Ile Leu Phe Met Leu Met Phe Ile Ser Ile Ala Val Thr Gly Ile Leu
 900 905 910

Glu Met Gln Trp Gly Gly Val Gly Ile Asp Asp Trp Trp Arg Asn Glu
 915 920 925
 Gln Phe Trp Val Ile Gly Gly Ala Ser Ser His Leu Phe Ala Leu Phe
 930 935 940
 Gln Gly Leu Leu Lys Val Leu Ala Gly Val Asn Thr Asn Phe Thr Val
 945 950 955 960
 Thr Ser Lys Ala Ala Asp Asp Gly Ala Phe Ser Glu Leu Tyr Ile Phe
 965 970 975
 Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Leu Leu Ile Ile Asn
 980 985 990
 Ile Ile Gly Val Ile Val Gly Val Ser Asp Ala Ile Ser Asn Gly Tyr
 995 1000 1005
 Asp Ser Trp Gly Pro Leu Phe Gly Arg Leu Phe Phe Ala Leu Trp Val
 1010 1015 1020
 Ile Val His Leu Tyr Pro Phe Leu Lys Gly Met Leu Gly Lys Gln Asp
 1025 1030 1035 1040
 Lys Met Pro Thr Ile Ile Val Val Trp Ser Ile Leu Leu Ala Ser Ile
 1045 1050 1055
 Leu Thr Leu Leu Trp Val Arg Val Asn Pro Phe Val Ala Lys Gly Gly
 1060 1065 1070
 Pro Val Leu Glu Ile Cys Gly Leu Asn Cys Gly Asn
 1075 1080
 <210> 25
 <211> 685
 <212> PRT
 <213> *Gossypium hirsutum*
 <400> 25
 Arg Arg Trp Val Pro Phe Cys Lys Lys His Asn Val Glu Pro Arg Ala
 1 5 10 15
 Pro Glu Phe Tyr Phe Asn Glu Lys Ile Asp Tyr Leu Lys Asp Lys Val
 20 25 30
 His Pro Ser Phe Val Lys Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu
 35 40 45
 Glu Phe Lys Val Arg Ile Asn Ala Leu Val Ala Lys Ala Gln Lys Lys
 50 55 60
 Pro Glu Glu Gly Trp Val Met Gln Asp Gly Thr Pro Trp Pro Gly Asn
 65 70 75 80
 Asn Thr Arg Asp His Pro Gly Met Ile Gln Val Tyr Leu Gly Ser Ala
 85 90 95
 Gly Ala Leu Asp Val Asp Gly Lys Glu Leu Pro Arg Leu Val Tyr Val
 100 105 110

Ser Arg Glu Lys Arg Pro Gly Tyr Gln His His Lys Lys Ala Gly Ala
 115 120 125
 Glu Asn Ala Leu Val Arg Val Ser Ala Val Leu Thr Asn Ala Pro Phe
 130 135 140
 Ile Leu Asn Leu Asp Cys Asp His Tyr Ile Asn Asn Ser Lys Ala Met
 145 150 155 160
 Arg Glu Ala Met Cys Phe Leu Met Asp Pro Gln Phe Gly Lys Lys Leu
 165 170 175
 Cys Tyr Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Arg His Asp
 180 185 190
 Arg Tyr Ala Asn Arg Asn Val Val Phe Phe Asp Ile Asn Met Leu Gly
 195 200 205
 Leu Asp Gly Leu Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Val Phe
 210 215 220
 Asn Arg Gln Ala Leu Tyr Gly Tyr Asp Pro Pro Val Ser Glu Lys Arg
 225 230 235 240
 Pro Lys Met Thr Cys Asp Cys Trp Pro Ser Trp Cys Cys Cys Cys Cys
 245 250 255
 Gly Gly Ser Arg Lys Lys Ser Lys Lys Lys Gly Glu Lys Lys Gly Leu
 260 265 270
 Leu Gly Gly Leu Leu Tyr Gly Lys Lys Lys Lys Met Met Gly Lys Asn
 275 280 285
 Tyr Val Lys Lys Gly Ser Ala Pro Val Phe Asp Leu Glu Glu Ile Glu
 290 295 300
 Glu Gly Leu Glu Gly Tyr Glu Glu Leu Glu Lys Ser Thr Leu Met Ser
 305 310 315 320
 Gln Lys Asn Phe Glu Lys Arg Phe Gly Gln Ser Pro Val Phe Ile Ala
 325 330 335
 Ser Thr Leu Met Glu Asn Gly Gly Leu Pro Glu Gly Thr Asn Ser Thr
 340 345 350
 Ser Leu Ile Lys Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu Glu
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 385 390 395 400
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 Asn Leu Ser Asp Arg Leu His Gln Val Leu Arg Trp Ala Leu Gly Ser
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Val Glu Ile Phe Leu Ser Arg His Cys Pro Leu Trp Tyr Gly Tyr Gly
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Gly Lys Leu Lys Trp Leu Glu Arg Leu Ala Tyr Ile Asn Thr Ile Val
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Tyr Pro Phe Thr Ser Ile Pro Leu Leu Ala Tyr Cys Thr Ile Pro Ala
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Val Cys Leu Leu Thr Gly Lys Phe Ile Ile Pro Thr Leu Ser Asn Leu
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His Gly Asn Arg Val His Pro Val Ser Leu Ser Asp Pro Thr Val Ala
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Tyr Gly Ser Val Ala Trp Lys Asp Arg Met Glu Glu Trp Lys Arg Lys
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Gln Asn Glu Lys Leu Gln Val Val Arg His Glu Gly Asp Pro Asp Phe
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁷ : C12N 15/54, 1/21, 9/10, C12Q 1/48, 1/68</p>	<p>A3</p>	<p>(11) International Publication Number: WO 00/04166 (43) International Publication Date: 27 January 2000 (27.01.00)</p>
<p>(21) International Application Number: PCT/US99/15871 (22) International Filing Date: 13 July 1999 (13.07.99) (30) Priority Data: 60/092,844 14 July 1998 (14.07.98) US (71) Applicant (for all designated States except US): E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): ALLEN, Stephen, M. [US/US]; 2225 Rosewood Drive, Wilmington, DE 19810 (US). FADER, Gary, M. [US/US]; 1000 Woods Lane, Landenberg, PA 19350 (US). FALCO, Saverio, Carl [US/US]; 1902 Millers Road, Arden, DE 19810 (US). KINNEY, Anthony, J. [GB/US]; 609 Lore Avenue, Wilmington, DE 19809 (US). LIGHTNER, Jonathan, E. [US/US]; 4180 Delta Road, Airville, PA 17302 (US). MIAO, Guo-Hua [CN/US]; 202 Cherry Blossom Place, Hockessin, DE 19707 (US). RAFALSKI, J., Antoni [US/US]; 2028 Longcome Drive, Wilmington, DE 19810 (US). THORPE, Catherine, J. [GB/GB]; 120 Ross Street, Cambridge CB1 3BU (GB).</p>	<p>(74) Agent: MAJARIAN, William, R.; E.I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US). (81) Designated States: AE, AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report. (88) Date of publication of the international search report: 27 April 2000 (27.04.00)</p>	

(54) Title: PLANT CELLULOSE SYNTHASES

(57) Abstract

This invention relates to an isolated nucleic acid fragment encoding a cellulose synthase. The invention also relates to the construction of a chimeric gene encoding all or a portion of the cellulose synthase, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the cellulose synthase in a transformed host cell.

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1
----- 60
SEQ ID NO:2 -----
SEQ ID NO:4 RAAGAGGNNKPPQREKGLASVSLP-LPHERITFFPFRRTERR---RYRACPG---I
SEQ ID NO:6 -----
SEQ ID NO:8 RESYTKRSRLAQFRAAFCAGPFF--ATAACACERSFPGDORAGGLAATCAAAAGTV
SEQ ID NO:10 -----
SEQ ID NO:12 RCS---KMTCSFFPTTAKLSPRATP
SEQ ID NO:14 -----
SEQ ID NO:16 -----
SEQ ID NO:18 -----
SEQ ID NO:20 -----
SEQ ID NO:22 -----
SEQ ID NO:23 -----
SEQ ID NO:24 NHTGGR
SEQ ID NO:25 -----
SEQ ID NO:26 NASTFPQTSKVVNNSGSGQVVKFAKATSBQKTVSLP-RDWIELGELGADYENTYVILP
SEQ ID NO:27 -----
SEQ ID NO:28 R---PR
SEQ ID NO:29 -----

61
----- 120
SEQ ID NO:2 -----
SEQ ID NO:4 W-RSGSARD---ME-ASAGLVAGSNNKELV-VIRNDSGFGKIP---NDRNSGVCQI--
SEQ ID NO:6 -----
SEQ ID NO:8 KERPAGRGGQFENE-ASAGLVAGSNNKELV-VIRNDSGSAAGDGNARRRACFCQI--
SEQ ID NO:10 -----
SEQ ID NO:12 -----
SEQ ID NO:14 -----
SEQ ID NO:16 -----
SEQ ID NO:18 -----
SEQ ID NO:20 -----
SEQ ID NO:22 -----
SEQ ID NO:23 -----
SEQ ID NO:24 -----
SEQ ID NO:25 -----
SEQ ID NO:26 FTFGQPMATZAREQTVNMLFTGFTVETVLAALAKVLDGQVTFHFGAGANRRCNSMFA
SEQ ID NO:27 -----
SEQ ID NO:28 -----
SEQ ID NO:29 -----

121
----- 180
SEQ ID NO:2 -----
SEQ ID NO:4 CQDQVEMFQSEFFVACISCAFFVCRCTETERRSSTGRCFCQKTRFKLMSCARVWSD
SEQ ID NO:6 -----
SEQ ID NO:8 CQDQVYGFQSEFFVACISCAFFVCRCTETERRSSTGRCFCQKTRFKLMSCARVWSD
SEQ ID NO:10 -----
SEQ ID NO:12 -----
SEQ ID NO:14 CQDQVGLVYDGLFVACISCEFFVCRCTETERRSSTGRCFCQKTRFKLMSCARVWSD
SEQ ID NO:16 -----
SEQ ID NO:18 -----
SEQ ID NO:20 -----
SEQ ID NO:22 -----
SEQ ID NO:23 CQDQVLAETSNVAVACISCAFFVCRCTETERRSSTGRCFCQKTRFKLMSCARVWSD
SEQ ID NO:24 -----
SEQ ID NO:25 -----
SEQ ID NO:26 CQDQVWDESDVWPC-SCATKICRCCFRDAGK-VGLCPKCRBYK

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FOR THE PURPOSES OF INFORMATION ONLY

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INTERNATIONAL SEARCH REPORT

International Application No
PC., US 99/15871

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/54 C12N1/21 C12N9/10 C12Q1/48 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 00549 A (WILLIAMSON RICHARD EDWARD ;PENG LIANGCAI (AU); ARIOLI ANTONIO (AU)) 8 January 1998 (1998-01-08) see SEQ ID NOs:1-12 --- -/--	1,2,4-7, 10-17

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

- * Special categories of cited documents :
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 - *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 - *O* document referring to an oral disclosure, use, exhibition or other means
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 - *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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 - *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
 - *Z* document member of the same patent family

Date of the actual completion of the international search
9 February 2000

Date of mailing of the international search report
23. 02. 00

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Maddox, A

INTERNATIONAL SEARCH REPORT

International Application No
PL., US 99/15871

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>ARIOLI, T., ET AL.: "Arabidopsis thaliana cellulose synthase catalytic subunit (RSW1) gene complete cds" EMBL ACCESSION NO:AF027172, 3 February 1998 (1998-02-03), XP002124282 the whole document</p>	1,4-6, 13-17
X	<p>-& ARIOLI, T. ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" SCIENCE, vol. 279, 30 January 1998 (1998-01-30), pages 717-720, XP002124283 the whole document & ARIOLI, T., ET AL.: "Cellulose synthase catalytic subunit" TREMBL ACCESSION NO:048946, 1 June 1998 (1998-06-01), ---</p>	6,13-17
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International Application No

PC, US 99/15871

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 18949 A (CALGENE INC ;PEAR JULIE R (US); STALKER DAVID M (US); DELMER DEBOR) 7 May 1998 (1998-05-07) figures 3A-C,6A-E,7A-D ---	1,2,4-7, 10-17
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X	-& PEAR, J.R., ET AL.: "HIGHER PLANTS CONTAIN HOMOLOGS OF THE BACTERIAL CELA GENES ENCODINGTHE CATALYTIC SUBUNIT OF CELLULOSE SYNTHASE" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, October 1996 (1996-10), pages 12637-12642, XP002061424	6,12-17
Y	see whole document particularly footnote left col page 12639	1,2,4-6
	& PEAR, J.R., ET AL.: "Cellulose synthase fragment" TREMBL ACCESSION NO:P93156, 1 May 1997 (1997-05-01), ---	
X	PEAR, J.R., ET AL.: "Gossypium hirsutum cellulose synthase (celA1) mRNA, complete cds" EMBL ACCESSION NO:U58283, 13 December 1996 (1996-12-13), XP002124439 the whole document	1,2,4-6, 13-17
X	-& PEAR, J.R., ET AL.: "HIGHER PLANTS CONTAIN HOMOLOGS OF THE BACTERIAL CELA GENES ENCODINGTHE CATALYTIC SUBUNIT OF CELLULOSE SYNTHASE" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, October 1996 (1996-10), pages 12637-12642, XP002061424 the whole document & PEAR, J.R. ET AL.: "Cellulose synthase" TREMBL ACCESSION NO:P93155, 1 May 1997 (1997-05-01), ---	6,13-17
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INTERNATIONAL SEARCH REPORT

International Application No
 PCT/US 99/15871

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WU, L., ET AL.: "Arabidopsis thaliana cellulose synthase mRNA, partial cds" EMBL ACCESSION NO: AF062485, 18 May 1998 (1998-05-18), XP002129995 the whole document</p> <p>-& WU, L., ET AL.: "AraxCelA, a new member of cellulose synthase gene family from Arabidopsis thaliana (accession no. AF062485) (PGR 98-113)" PLANT PHYSIOLOGY 117:1125, July 1998 (1998-07), XP002130048</p> <p>---</p>	7,10-17
X	<p>DBEST DATABASE ID:37681, 2 December 1993 (1993-12-02), XP002124440 the whole document</p> <p>& SASAKI, T.: "Rice cDNA, partial sequence (R1814-1A)" EMBL ACCESSION NO:D24381, 29 November 1993 (1993-11-29),</p> <p>---</p>	1,2,4-6, 16,17
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Y	<p>SASAKI, T., ET AL.: "Rice cDNA, partial sequence (R2668_1A)" EMBL ACCESSION NO:D24862, 29 November 1993 (1993-11-29), XP002124441 the whole document</p> <p>-& DBEST DATABASE ID:38158, 2 December 1993 (1993-12-02), XP002124442 the whole document</p> <p>---</p>	1,2,4-6
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INTERNATIONAL SEARCH REPORT

International Application No
 PL., US 99/15871

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 00551 A (MAYO FOUNDATION ;MCDONALD JOHN A (US); SPICER ANDREW P (US); AUGUS) 8 January 1998 (1998-01-08) see page 14 lines 21 and 22, and SEQ ID NO:48	6
P,X	--- EP 0 875 575 A (NISSHIN SPINNING) 4 November 1998 (1998-11-04) the whole document	1,2,4-7, 10-12
P,X	--- SASAKI, T., ET AL.: "Oryza sativa cDNA, partial sequence (R2825 6A)." EMBL ACCESSION NO:AU031954, 19 October 1998 (1998-10-19), XP002129999 the whole document	16,17
P,X	--- TAYLOR, N.G., ET AL.: "Arabidopsis thaliana cellulose synthase catalytic subunit (IRX3) mRNA, complete cds." EMBL ACCESSION NO:AF088917, 25 May 1999 (1999-05-25), XP002130000 the whole document	1,2,6, 16,17
P,X	--- BLEWITT, M., ET AL.: "BNLGH13827 Six-day Cotton fiber Gossypium hirsutum cDNA 5' similar to (AF027172) cellulose synthase catalytic subunit [Arabidopsis thaliana] gi 4049343 gnl PID e1361041 (AL034567) cellulose synthase catalytic subunit (RSW1) [Arabidopsis thaliana], mRNA sequence." EMBL ACCESSION NO:A1729626, 12 June 1999 (1999-06-12), XP002130001 the whole document	1,2,6, 16,17
P,X	--- BLEWITT, M., ET AL.: "BNLGH15835 Six-day Cotton fiber Gossypium hirsutum cDNA 5' similar to (AF027172) cellulose synthase catalytic subunit [Arabidopsis thaliana] gi 4049343 gnl PID e1361041 (AL034567) cellulose synthase catalytic subunit (RSW1) [Arabidopsis thaliana], mRNA sequence." EMBL ACCESSION NO:A1729981, 12 June 1999 (1999-06-12), XP002130002 the whole document	1,2,6, 16,17
P,X	--- LAOSINCHAI W., ET AL.: "Gossypium hirsutum cellulose synthase catalytic subunit (celA3) mRNA, complete cds." EMBL ACCESSION NO:AF150630, 21 June 1999 (1999-06-21), XP002130003 the whole document	1,2,6, 16,17
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INTERNATIONAL SEARCH REPORT

International Application No
PC, US 99/15871

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 723 764 A (SINGLEARY GEORGE WILLIAM ET AL) 3 March 1998 (1998-03-03) ---	13
A	AMOR Y ET AL: "EVIDENCE FOR A CYCLIC DIGUANYLIC ACID-DEPENDENT CELLULOSE SYNTHASE IN PLANTS" PLANT CELL,US,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 3, page 989-995 XP002061420 ISSN: 1040-4651 the whole document ---	6,18
A	LI ET AL: "beta-Glucan synthesis in the cotton fiber" PLANT PHYSIOLOGY,US,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 101, no. 4, page 1149-1156 XP002087180 ISSN: 0032-0889 the whole document ---	6,18
A	WO 91 13988 A (UNIV TEXAS) 19 September 1991 (1991-09-19) the whole document -----	1-6, 13-18

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/ 15871

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheets

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-6,13-18 all partially

Nucleic acid fragments encoding barley cellulose synthase, and corresponding polypeptide as represented by SEQ ID NOs:1 and 2, fragments encoding amino acid sequence 90% identical thereto, transformed hosts containing and methods for obtaining said sequences, methods for altering expression and methods for evaluating inhibitors using said sequences.

2. Claims: 1-6,13-18 all partially and 7-12 all completely

Nucleic acid fragments encoding corn cellulose synthase, and corresponding polypeptide as represented by SEQ ID NOs:3-10, fragments encoding amino acid sequence 90% identical thereto, transformed hosts containing and methods for obtaining said sequences, methods for altering expression and methods for evaluating inhibitors using said sequences.

3. Claims: 1-6,13-18 all partially

Nucleic acid fragments encoding rice cellulose synthase, and corresponding polypeptide as represented by SEQ ID NOs:11 and 12, fragments encoding amino acid sequence 90% identical thereto, transformed hosts containing and methods for obtaining said sequences, methods for altering expression and methods for evaluating inhibitors using said sequences.

4. Claims: 1-6,13-18 all partially

Nucleic acid fragments encoding soybean cellulose synthase, and corresponding polypeptide as represented by SEQ ID NOs:13-18, fragments encoding amino acid sequence 90% identical thereto, transformed hosts containing and methods for obtaining said sequences, methods for altering expression and methods for evaluating inhibitors using said sequences.

5. Claims: 1-6,13-18 all partially

Nucleic acid fragments encoding wheat cellulose synthase, and corresponding polypeptide as represented by SEQ ID NOs:19-22, fragments encoding amino acid sequence 90% identical thereto, transformed hosts containing and methods for obtaining said sequences, methods for altering expression and methods for evaluating inhibitors using said sequences.

6. Claim : 18 partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Method for evaluating a compound for inhibitory activity on cellulose synthase comparing activity of cellulose synthase produced in a transformed host with and without the addition of the compound, not covered by any of the previous groups of claimed inventions 1-5.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PL, US 99/15871

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9800549 A	08-01-1998	AU 3160397 A CA 2259126 A EP 0956353 A	21-01-1998 08-01-1998 17-11-1999
WO 9818949 A	07-05-1998	AU 5092398 A BR 9712457 A EP 0938573 A	22-05-1998 19-10-1999 01-09-1999
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